AUTOMATED INSTRUMENTATION FOR CLINICAL AND RESEARCH LABORATORIES

Innovations and Development of Vertical Light Beam Photometers and Electronic Pipettes

OSMO SUOVANIEMI

Department of Medical Chemistry, Department of Clinical Chemistry,
University of Helsinki and
Biohit Oy, Helsinki

AUTOMATED INSTRUMENTATION FOR CLINICAL AND RESEARCH LABORATORIES

Innovations and Development of Vertical Light Beam Photometers and Electronic Pipettes

Osmo Suovaniemi

Academic dissertation

To be publicly examined by permission of the Medical Faculty of the University of Helsinki, in the small auditorium at Siltavuorenpenger 10, Helsinki, on 4th February 1994, at 12 o'clock noon.

Helsinki 1994

CONTENTS

SUMMARY 5

AIMS OF THE PRESENT WORK 6

i-iiii

A. PHOTOMETRIC METHODS IN CLINICAL AND RESEARCH CHEMISTRY 7

A1. HISTORICAL SURVEY AND PRESENT TRENDS 7

A1.1. The Duboscq colorimeter and other early developments 7

A1.2. Enzyme-Immuno-Assay 8

A1.3. Measurement of light-scattering 8

A1.4. The trend towards automation 9

A2. THEORY OF VERTICAL PHOTOMETRY 9

A2.1. Introduction 9

A2.2. The Bouguer-Lambert-Beer Law 10

A2.3. Horizontal versus vertical light beams 10

A3. INSTRUMENT DESIGN 11

A3.1. Optical design of the FP-901 Analyzer 13

A3.2. Electronics and mechanics of the FP-901 Analyzer 17

A4. TEST RESULTS AND APPLICATIONS 18

A4.1. Sources of errors in photometry 19

A4.2. Evaporation and temperature 19

A4.3. Inaccurate pipetting of solute 20

A4.4. Layering 21

A4.5. Effect of the shape of the liquid surface in cuvettes made of polystyrene 23

A4.6. Performance of vertical light beam photometers 25

A5. CONCLUSIONS 28

B. LIQUID DISPENSION WITH ELECTRONIC PIPETTES 29

B1. FORERUNNER: THE MECHANICAL PIPETTE 29
B2. DEVELOPMENT OF ELECTRONIC PIPETTES 30
B3. THE BIOHIT PROLINE ELECTRONIC PIPETTE 31
B4. TEST RESULTS 35
B4.1. Precision and accuracy of pipetting 36
B4.2. Multichannel pipetting 40
B4.3. Pipetting speed 42
B5. CONCLUSIONS 42

C. GENERAL DISCUSSION 43

D. FUTURE PROSPECTS 46

E. ACKNOWLEDGEMENTS 47

F. REFERENCES 48

APPENDICES I - VI

SUMMARY

Analytical photometers were designed and built, based on the principle of a vertical light path. With this principle the absorbance of large numbers of samples can be measured in a short time with exceptional precision and accuracy. The advantages and disadvantages of such photometers are discussed and test results are reported. Some methodology is presented to illustrate the practical use of the developed theory in general and the designed instrumentation in particular. Especially promising areas of application are endpoint and kinetic determinations of enzymatic reactions in clinical chemistry and biochemistry. Other fields particularly well-suited for measurements by vertical photometry include enzyme immunoassays and various applications related to the growth of bacteria. However, versatility and the specific advantages of vertical optics are not limited to these examples that have so far been assessed in practise. Further possible uses of the principle of vertical light paths are discussed.

The pipette is the most common device in laboratory liquid handling. An electronically controlled pipette was designed and built to minimize human pipetting error, yet enabling the handling of a large number of liquid samples with sufficient accuracy and precision. In the pipette design special attention was paid to ergonomic aspects of the pipetting procedure, by minimizing the weight of the device and by optimizing its shape and construction. Test results are reported with this device, confirming the requirements of precision and accuracy.

AIMS OF THE PRESENT WORK

i. To develop the theory for vertical photometry

ii. To develop automated photometers with a vertical light path so that large numbers of samples can be measured in a short time and to develop new measuring methods based on vertical photometry for research and routine laboratories.

iii. To develop accurate and precise pipetting devices for research and routine laboratories that enable liquid handling with a large number of samples, and with minimized human error and physical stress.

iiii. To test the aforementioned devices for their accuracy and precision.

A. PHOTOMETRIC METHODS IN CLINICAL AND RESEARCH CHEMISTRY

A1. HISTORICAL SURVEY AND PRESENT TRENDS

A1.1. *The Duboscq colorimeter and other early developments*

Colorimetric methods came into general use in clinical chemistry shortly after the turn of the century. In 1904, Folin introduced a simple and accurate method for determining creatinine in urine (Folin, 1904), which gave great impetus to the more general development of colorimetric methods (Caraway, 1981). In his procedure Folin used a colorimeter first introduced in 1854 by Jules Duboscq (Myers, 1924; Caraway, 1981). In its original form this so-called Duboscq colorimeter was a visual instrument, as were the microspectroscopes used by MacMunn (1885) and Keilin (1925) in their original descriptions of respiratory pigments in tissues and cells (see also Keilin, 1966). However, with the Duboscq colorimeter (Fig. 1) the operator compared and matched unknown and standard coloured solutions visually (Kober, 1928; Freund, 1932; Krebs, 1935; Dawes, 1972), varying the pathlength of transmitted light. The pathlength was adjusted with a solution of unknown concentration until the visual intensity of transmitted light equalled that of a standard solution. In this case, the concentration of the unknown solution could be obtained from the relationship (see Fig. 1)

$$(1) \qquad l\ c = l_x\ c_x$$

where c and c_x are the concentrations of standard and unknown solutions, respectively, and l and l_x the corresponding pathlengths.

Numerous colorimetric instruments have been introduced since the pioneering Duboscq device (Myers, 1924; Kober, 1928;

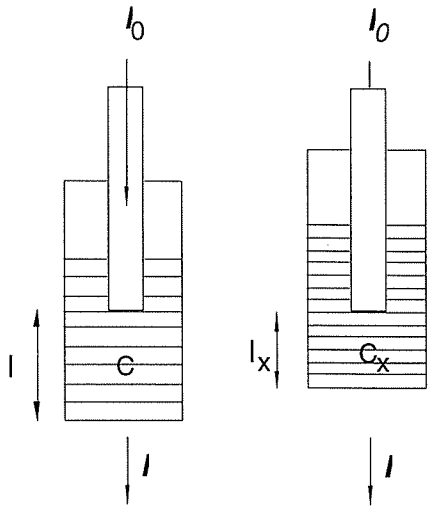

Figure 1. With the principle of the Duboscq colorimeter the length of the light path through the liquids can be adjusted so that the intensities of the transmitted light are equal.

Mellon, 1939; Dawes, 1972). Some of the later developments, in particular the Klett-Summerson photoelectric colorimeter (in 1939) and the classical Beckman DU spectrophotometer (in 1941), were as significant for advancing the methodology of both routine clinical and biochemical research work as were the microspectroscope and the Duboscq colorimeter nearly a century earlier. The continuously balancing double-beam recording spectrophotometer described by Cary (Cary, 1949), and the dual-wavelength spectrophotometers developed by Chance (Chance, 1951) have also had a strong impact, especially in biochemical and biophysical research work. Descendants of both these instruments are still common in research laboratories today, though considerably modernized by present-day electronic and microprocessor technology.

The evolution of clinical enzymology has been thoroughly discussed by Büttner (1981). It was strongly related to the history of basic enzymological research, and in particular to the development by Michaelis and Menten (1913) of their classical theory of enzyme kinetics. The development of the universally applicable principle of the optical test by Warburg et al. (1935) and the standardization of enzyme activity (Intl. Union Biochem., 1961) have, likewise, been essential for a unified quantitative determination of enzyme activities in clinical chemistry. Determination of enzyme activities as a quantitative diagnostic method has since become very widely used in clinical laboratories worldwide. Much effort has been exerted, not only in the design of instrumentation, but also in developing suitable reagents and standards, including pure substrates, enzyme preparations as well as their combinations in complete "kits" for specific analytical purposes.

A1.2. *Enzyme-Immuno-Assay*

Immunological methodology, based on specific antibody-antigen recognition, is the latest newcomer to the clinical-analytical laboratory. This has become a vast area of routine laboratory activity in the last 25 years. It has stimulated the development of a large variety of assays that all take advantage of the wide-ranging specificity and tight complexation of antibodies with their respective antigens (see Leinikki & Pässilä, 1976, Keller, 1978; Cappel et al., 1978; Lehtonen, 1982; Al Moudallal et al., 1984). Of these, the enzyme-linked immunosorbent assay (ELISA) or, simply, enzyme immunoassay (EIA; Engvall & Perlman, 1971; Van Weemen & Schuurs, 1971), has found the most widespread applications, especially in determining specific antibodies associated with a large variety of infectious diseases (see e.g. Ukkonen et al., 1977; Cappel et al., 1978; Hautanen et al., 1978; Leinikki et al., 1978; Granfors, 1979; Gripenberg et al., 1978, 1979; Iivanainen et al., 1979; Molnar et al., 1979; Milatovic & Braveny, 1980; Granfors et al., 1980; Mondesire et al., 1981; Salonen & Vaheri, 1981; McArthur and Sengupta, 1981; Kendall et al., 1983). These methods also, as so many others in clinical chemistry, are based on the photometric determination of the rate or extent of development of a coloured end product of an enzymatic reaction (see e.g.Lehtonen, 1982).

The development of the techniques for producing monoclonal antibodies (Köhler and Milstein, 1975) has also further advanced the field of immunological diagnosis, especially by increasing the specificity of the antibody-antigen reaction and thus diminishing the frequency of false positive results (see e.g. Jemmerson et al., 1982; Lansdorp et al., 1982; Dwyer et al., 1983).

A1.3. *Measurement of light-scattering*

Apart from the determination of concentrations of coloured substances by the absorption of monochromatic light, many photometers can also be used for the determination of liquid turbidity by light–scattering analysis. Liquid-phase immunoprecipitation analysis, for example, can be performed using photometers constructed specifically for measurements of light-scattering. This method is especially useful in measuring the turbidity of immune aggregates or of suspensions of individual proteins (see Deverill & Reeves, 1980). The turbidity of cell suspensions can also be monitored photometrically as a measure of cell growth (cell density). This can in some instances be applied to determine the effects of toxic or mutagenic substances on cell growth (see section A3), and adhesion and agglutination of cells.

Photometric methods thus continue to dominate the field of routine clinical chemistry in the determination of metabolites, toxins, proteins (including antibodies and antigens), and enzyme activities. Enzymatic methods have increasingly replaced older, less specific and less sensitive analytical tests of metabolite and antibody concentrations, and this appears to be a continuing trend.

A1.4. *The trend towards automation*

The successful development of photometric techniques and their application to diagnostic methods in routine clinical chemistry vastly increased the number of requested patient tests. This, in turn, caused increasing demands for simplification, automation and greater efficiency in the diagnostic procedures, to make them capable of handling the large demand. Among the first developments in this direction was the "continuous flow" system invented by Leonard Skeggs (Skeggs, 1957) and put into production by the Technicon Corporation in the mid 1950s. After the introduction of this concept, high-capacity clinical analyzers based on the continuous flow principle have dominated routine clinical chemistry laboratories. However, indications today suggest that smaller-sized discrete analyzer systems may partly or perhaps completely replace the bulky and less versatile continuous flow analyzers. In contrast to the latter, the new generation of analyzers (see section A3) process samples batchwise, i.e. determine the same components from several samples, or alternatively, several components by random access from several patient samples.

It has become increasingly clear that the use of large automatized analyzer systems has also had adverse effects. Because of their lack of versatility, they have induced various degrees of methodological conservatism. Thus, newly developed methods, more highly specific than older established ones, have often remained unimplemented, or their use has been delayed, because it is difficult and time-consuming to modify analytical procedures of large analyzers (Keller, 1978). The development of smaller and more versatile analyzer systems has been greatly assisted by the rapidly developing microprocessor technology. The large central computer of the hospital is being replaced in many instances by small microcomputers, which control the functions of the analyzers of the present-day generation.

It is intended in this present work to provide the background for some essential aspects in the development of the present-day automated photometric analyzers for clinical chemistry and immunological analysis. This work involves the author´s development of the first modern photometers, utilizing the principle of a vertical light path (Suovaniemi, 1976 and 1984, U.K. Patent 1,499,414, U.S. Patents 4,144,030 and 4,439,039, Suovaniemi and Järnefelt, 1982 and Appendices I-III). Others all over the world have subsequently adopted this principle, and at present the function of most photometric analyzers in clinical laboratories is based on it (Kurstak, 1986). The details of this development will be related in a historical perspective, the performance of these instruments will be critically assessed and further applications for research and routine use will be discussed on the basis of the test results.

A2. THEORY OF VERTICAL PHOTOMETRY

A2.1. *Introduction*

Until the 1970s, virtually all photometric measurements were done using a horizontally directed beam of light, which passes the sample holder (cuvette), followed by a light detector, signal amplification, and registra-

tion (horizontal measurement principle). The research work of the author began in 1968, and based on that work, the first patent application concerning photometric measurements with a vertically directed light beam was filed on 14 November 1973. Subsequently, this work led to several patents, patent applications and reports on methods and instruments related to the vertical measurement principle and its theory (Suovaniemi, 1976 and 1984, U.K. Patent 1,499,414, U.S. Patents 4,144,030 and 4,439,039, Canadian Patent 1,031,183; Suovaniemi & Järnefelt, 1982, Appendices I-III). At the present time, photometric analyzers of various kinds in clinical laboratories world-wide are largely based on this principle (Kurstak, 1986), the advantages and disadvantages of which will be discussed below.

A2.2. *The Bouguer-Lambert-Beer Law*

The classical work of Bouguer (1729) and Lambert (1790) led to the fundamental relationship between incident and transmitted radiant power (P_0 and P, respectively), and length of the optical path (b), at unit concentration of a light-absorbing substance, viz.

(2) $P/P_0 = T = e^{-kb}$

where T is the fractional light transmission and k is a specific absorbance, the absorption coefficient. This is equivalent to

(3) $-\ln T = k\,b$

Of much greater interest is the dependence of radiant power on the concentration of the absorbing species in solution (Beer 1852).

(4) $\log P_0/P = a\,b\,c$

a = specific absorptivity [l g^{-1}]
b = path length [cm]
c = concentration [$l^{-1}g$]

or

(5) $\log P_0/P = \varepsilon\,b\,c_m$

ε = molar absorptivity [l mol^{-1}]
b = path length [cm]
c_m = molar concentration [$l^{-1}mol$]

Absorbance, A, is the base-ten logarithm of the reciprocal of the transmittance as follows

(6) $A = \log P_0/P = -\log T$

leading to

(7) $A = a\,b\,c$

or

(8) $A = \varepsilon\,b\,c_m$

A2.3. *Horizontal versus vertical light beams*

This expression of the Bouguer-Lambert-Beer law (eqn 8) refers to the classical optical arrangement where the absorbance of a solution placed in a cuvette is measured by a beam of light entering the cuvette from one side, and leaving it from the other (see e.g. Kenner, 1973). Since the light beam enters the cuvette at right angles to its vertical axis, such measurements are referred to as horizontal. As eqn. 8 shows, in such a situation the measured absorbance is proportional to the concentration of the dissolved substance; increasing the amount of substance by increasing its volume does not affect the absorbance in a horizontal measurement.

Consider now the situation depicted in Fig. 2, where the light beam enters the cuvette along its vertical axis, and leaves the cuvette through the surface of the solution into the light detector. Alternatively, the measuring beam may pass in the opposite direction, first through the free surface of the solution, and leaving through the bottom of the cuvette. This situation may be called the vertical measurement principle. Obviously,

equation 8 applies as for the horizontal measurement, but only when the solution remains homogeneous. Indeed, homogeneity of the sample is a prerequisite for standard horizontal optical absorption measurements. Inaccurate pipettings of solvent and evaporation cause errors in horizontal measurements.

Consider, in contrast, that the sample in Fig. 2 is inhomogeneous, with separate layers of solution at different local solute concentration. To calculate absorbance we integrate along the vertical axis (z) and get

(9) $A = \int_0^b \varepsilon\, c\, dz$

where c = the z-dependent concentration and z=the vertical coordinate with the origin at the bottom of the cuvette.

In each element of volume

(10) $c = dm/dV$

where m is the mass of the absorbing substance and V is the volume. Referring to Fig. 2

(11) $dV = S\, dz$

where S is the cross-sectional area of the cuvette. Introducing eqns. (10) and (11) into eqn. (9) yields

(12) $A = \int_0^b \varepsilon\, (dm/S\, dz)dz = \varepsilon/S \int_0^b dm$

Since the total mass of the absorbing substance is always contained within the limits 0 and b, i.e. within the height of the solution, then independently of the vertical distribution of absorbing substance, and independently of the amount of solvent in the cuvette

(13) $A = m\, (\varepsilon/S)$

where the mass of the absorbing substance is expressed in moles.

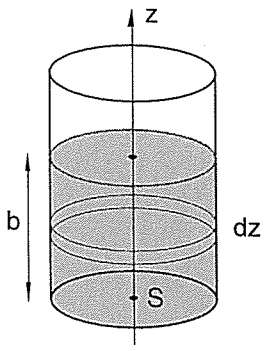

Figure 2.

This is an intriguing expression, inasmuch as it predicts that the absorbance now purely depends on how many moles of dissolved substance are in the cuvette, and not on its concentration, as in the horizontal measurement. This means, for example, that evaporation of solvent, or pipetting errors in solvent dispension, should have no effect on the absorbance measurement, since both cause variations in solute concentration but not in molar amount. Furthermore, and most importantly, the measured absorbance should be proportional to the total molar mass (amount) of the absorbing substance irrespective of its vertical distribution in the cuvette. These predictions from eqn. 13 will be tested experimentally below (see section A4).

A3. INSTRUMENT DESIGN

The increasing demand for larger sample handling capacity and volumes, especially in clinical chemistry, and above all for the rapidly developing field of immunochemical analysis in the late 1970s and 1980s, forcefully challenged instrumental development in a new way. Rapid photometric analysis of a large number of samples would clearly be very difficult using ordinary photometers, simply due to mechanical problems resulting

from the horizontal measurement principle. This problem might be solved using the vertical measurement principle, the theory of which was described above. In this case it should be relatively simple, in principle, to arrange a large number of samples next to one another in a matrix, and to direct a beam of light vertically through the bottom of each sample vessel at the time of measurement. The measurement of several samples could then be achieved either (i) by moving the sample matrix horizontally, or (ii) by moving the light beam from one sample to another across a fixed "cuvette" matrix. Correspondingly, it would also necessitate a mechanical-optical arrangement where the detector of the transmitted light would either (i) remain stationary above a stationary light beam or (ii) move in synchrony with the light beam. This would, furthermore, require the development of optically suitable and uniform "cuvette" matrices (see test results in section A4).

At the beginning of the 1970s, only one photometric instrument, a centrifuge photometer, was available. In this instrument, the optical light-path was parallel to the long axis of the cuvette, as a horizontal light beam would have been mechanically awkward. Photometric determinations with centrifugal analyzers differ from those with static photometers in that with the former, the absorbances are measured more or less simultaneously in a large number of cuvettes in rapid succession. The cuvettes are arranged radially on a rotor turning at relatively high speed – approximately 1000 rpm. During rotation, the cuvettes cross the light beam from lamp to detector. The samples and reagents intended to react with one another are transferred into the various cuvettes by centrifugal force. The arrangement of the radially-disposed cuvettes and of the transfer disc into which the samples are pipetted varies with different types of centrifugal analyzers. In the CentriChem system, the Gemsaec and the Rotochem, the samples and reagents are propelled from a transfer disc into the cuvettes arranged radially around the disc (cuvette ring). By contrast, the transfer disc and cuvette ring of the Gemeni II, the IL Multistat III, and the CobasBio form one disposable unit (Eisenwiener and Keller, 1979). However, the advantages of the vertical principle for increasing sample capacity and automation were overlooked.

After early developments of cuvette matrices for 9 samples and filter photometers using these (Fig. 3), it became increasingly clear that nine samples were not sufficient, and further, that if these instruments were to become of widespread use internationally, a more widely used and generally accepted matrix had to be employed. The so-called microtitration plate in common use by serologists for some time appeared to fulfil both these basic requirements. However, this plastic plate, typically with a matrix of 8 x 12, or 96, reaction wells was not originally developed for photometric measurements.

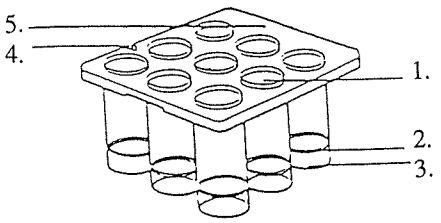

Figure 3. The FP-Analyzer Cuvette Block.
1. Nine cuvettes
2. Flat optical window
3. Protective rim
4. Groove, which ensures the correct position in the cuvette block tray
5. Place for identification

Thus its design and optical properties had to be developed for the present more demanding purpose (see Fig. 4).

Apart from the motif of using vertical photometry for the sake of large numbers of samples and automation, another advantage of this geometry became evident from the analysis described in section A2. Due to this geometry, it may be possible to avoid errors due to solute pipetting as well as evaporation. Furthermore, it has been predicted that the unique properties of the vertical measurement principle might lead to new dynamic applications of photometry in which the measured reaction does not lead to a homogeneous distribution of the measured absorbing substance (analyte) in the measuring cuvette. Examples of such possibilities are, the agglutination reactions typical in blood group determinations, other serological reactions, determination of blood clotting, and even the growth of cells by measurement of turbidity (Suovaniemi, U.S. Patents 4,290,997 and 4,452,902, Suovaniemi et al., 1984).

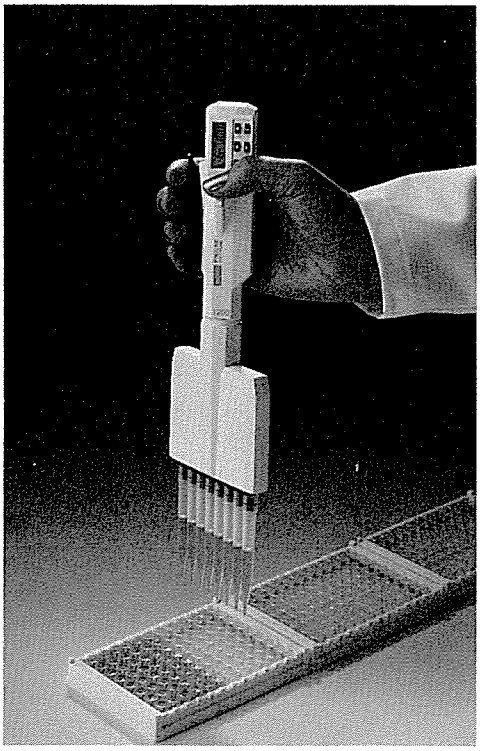

Figure 4. Biohit microstrips (1x8 wells) in a plate (8x12 wells) with the Biohit Proline Electronic Pipette.

A3.1. *Optical design of the FP-901 Analyzer*

The principle of handling several samples and reagents simultaneously in colorimetric assays led to the development of several instruments in which the use of the vertical light beam principle is essential. Here, the FP-901 Analyzer is described in some detail, as a representative example of this development. The optical designs of the Multiskan, Mutascreen, Bioscreen and Auto-EIA Analyzers are presented as well.

The FP-901 is a nine-channel photometer, designed to measure the absorbances of nine samples simultaneously, both in the visible and in the ultraviolet ranges. The construction differs fundamentally from that of ordinary photometers, because the direction of the measuring light beam is vertical. The measuring ray enters through the bottom of the cuvette, and passes through the free surface of the liquid in the cuvette, after which it reaches the detector. The theoretical principles of such a measurement design were discussed above (section A2).

Fig. 5 shows the optical system of the FP-901 Analyzer. A halogen lamp provides the light, which passes through a light chopper and is deflected by a dichroic mirror (a UV mirror) to eliminate a large proportion of the long–wavelength radiation. Two major advantages are achieved with this feature. The excess heat is eliminated, which minimizes the heat exchange between the interference filter and sample. The mirror also levels the visible spectrum and thus facilitates a broader usable spectral range. The light then passes through an appropriate interference filter, which is one of eight filters

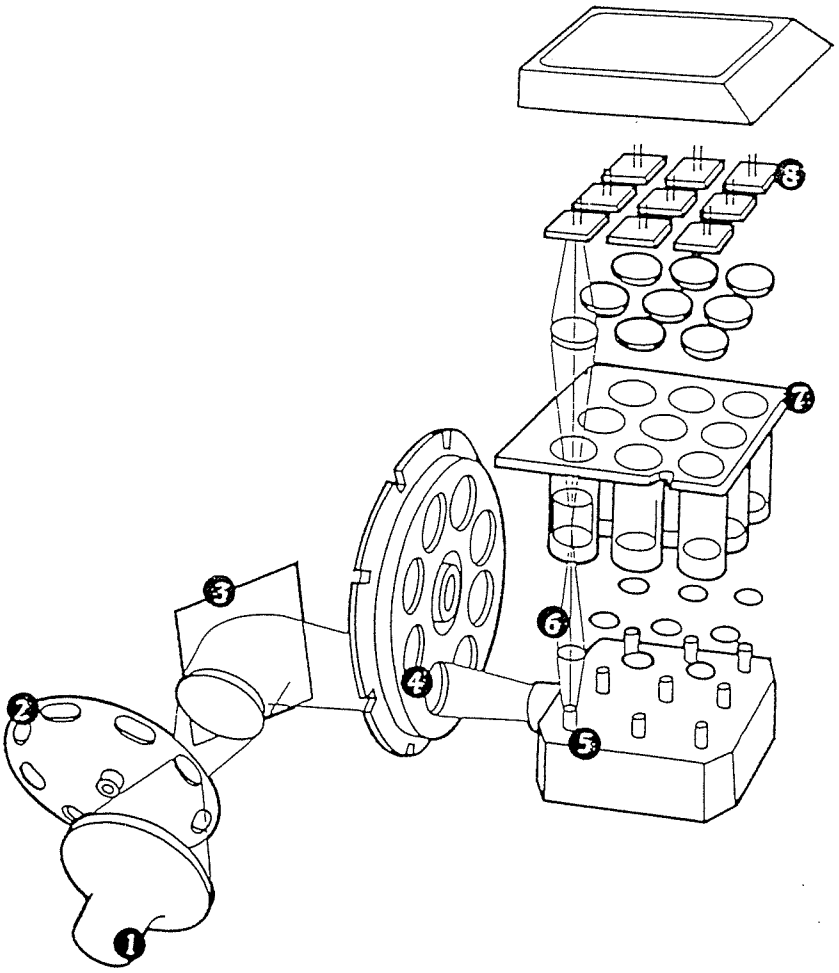

Figure 5. The optical system of the FP-901 Analyzer. A halogen lamp (1) provides the light, which passes through a chopper (2) and is deflected by a UV-mirror (3). The light then passes through the appropriate interference filter (4) and enters the fiber bundle (5), which divides the light into nine channels, and deflects the light upwards. The nine light beams (6) emerging vertically, are collimated by a lens and pass through the cuvettes (7). The light is then focused on the silicon photodetectors (8) by another lens between the cuvette and the detector.

arranged in a filter wheel, and enters the optical fibre bundle. The latter divides the light into nine channels and deflects the light upwards. The nine light beams emerge vertically, are collimated by a lens, and pass through the cuvettes. The light is then focussed on the silicon photodetectors by another lens. The detector head is flushed with air to prevent condensation of water on the detector lenses.

Multiskan MC is a further development of the FP-901 system and Titertek Multiskan,

which in turn were designed according to the basic principles of the FP-9 system. The goal was to make possible automatic, photometric evaluation of reactions performed in microtitration plates (see Figure 4). The feature of multichromatic (MC) measurement has been incorporated in the Multiskan MC so that measurements from the same sample at two or more different wavelengths are possible. This is accomplished by the instrument's microprocessor-directed programmable operation, which selects the desired wavelengths by rotating the filter wheel and by taking measurements in succession. By using measurements at two or more different wavelengths, it is possible to eliminate nonspecific absorbances due to turbidity or other side effects in the reaction mixture.

The FP-901 photometer has an identical filter wheel, making measurements at several wavelengths possible. It should be emphasized that measurements at several wavelengths will allow analysis of several parameters simultaneously, or alternatively, the elimination of the effect of interfering nonspecific substances.

The design of the Multiskan MC is basically similar to that of the FP-901. It is provided with a carrier for the microtitration plate (see Figure 5a). Its optical system features eight channels in linear array, corresponding to the row of eight wells in the

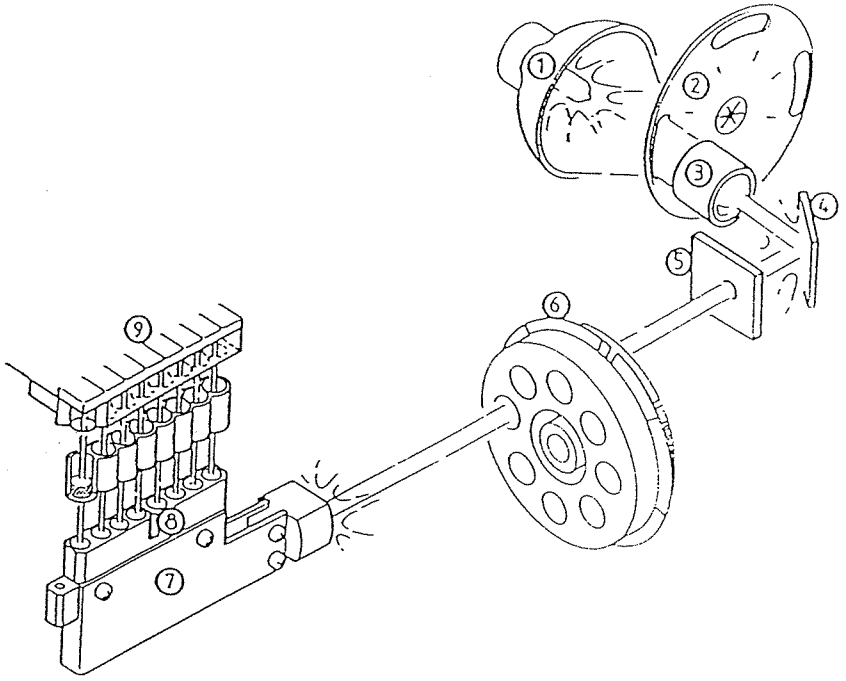

Figure 5a. The optical system of the Multiskan Analyzer.

1. Halogen lamp
2. Chopper wheel
3. Aperture and condenser lens
4. Dichroic mirror
5. Aperture
6. Interference filter (filter wheel)
7. Optical fibre bundle
8. Focusing lenses
9. Upper lenses and silicon photodetectors

microtitration plate. The absorbances of each row are measured simultaneously, after which the plate is moved and the next row measured. At each step the measurement can be performed at one or several wavelengths.

A large number of methods have been introduced for the Multiskan system. The system has become especially popular in enzyme immunoassays, as shown by the large number of applications published (see Section A1.2. and Ruitenberg et al., 1980; Vaheri et al., 1980; Douillar et al., 1981; Uotila et al., 1981).

The availability of multichannel pipettes developed for use with the 8x12 microtitration plates (see Section B) considerably improves the versatility of the Multiskan systems.

The optical systems of the FP-9, FP-901 and Multiskan Analyzers have been designed to collect all light passing through the cuvette to the silicon photodetector. For this purpose selection of the detector´s light sensitive surface area has been as wide as possible.

In photometry, in general, the specific absorbance value depends on the bandwidth, blocking value and the number of layers of the interference filter and also on the age of the filter itself (Manual of Optical Filters and Coatings, Corion Corporation, 6-7, 1983).

The FP-9, FP-901 and Multiskan Analyzers have their own removable interference filters (340-750 nm) with the halfbandwidth of 4-10 nm. The combination of the IR-light

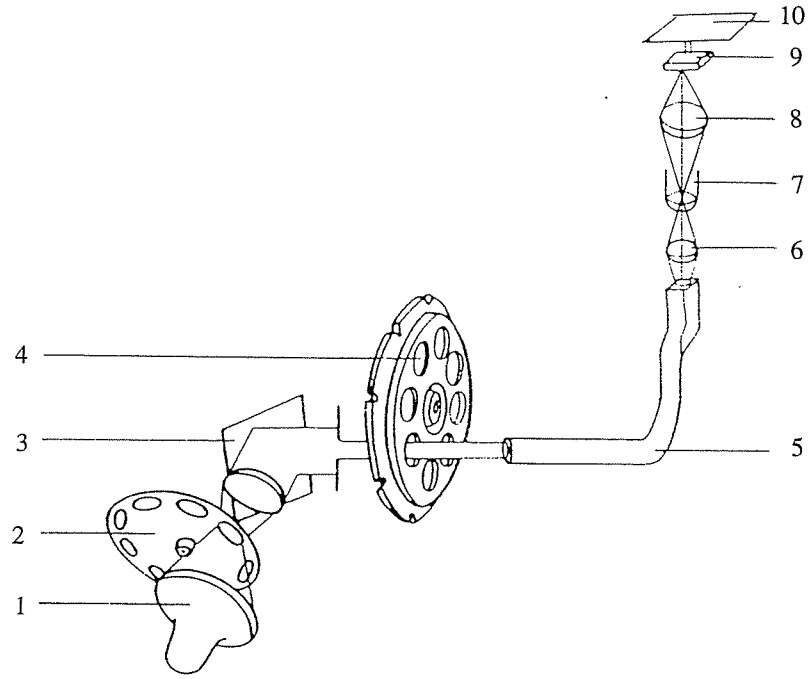

Figure 5b. Optical system of the Mutascreen, Bioscreen and Auto-EIA Analyzers.

1. Halogen lamp
2. Chopper wheel
3. Dichroic mirror
4. Interference filter (filter wheel)
5. Optical fibre bundle
6. Focusing lens
7. Cuvette (well)
8. Focussing lens
9. Silicon photodetector
10. Preamplifier

cutting dichroic mirror and the interference filter with high blocking (10^{-6}–10^{-7}) screens out the passage of second and higher order wavelengths. Consequently, the amount of stray light is insignificant in practise (Appendix III).

Figure 5b. shows the optical system of the Mutascreen, Bioscreen and Auto-EIA Analyzers, which are discussed.

The matrix of the 8x12, or 96, reaction wells (see Figure 5b), located on the X-carriage, moves in the X-direction with a stepping motor. The upper end of the flexible optical fibre bundle and the focussing lens are attached to the lower arm of the Y-carriage. The higher arm of the Y-carriage contains the focussing lens, silicon photodetector and preamplifier. The Y-carriage assembly moves in the Y-direction with a stepping motor. Consequently, the same optical system measures all reaction wells of the 8x12 well matrix. This optical system provides more light for measurement compared to the 8- or 9-channel fibre bundles (see Figure 5 and 5a), and there are no errors due to the differences of optical components.

A3.2. *Electronics and mechanics of the FP-901 Analyzer*

The FP-901 is a microprocessor-controlled instrument (see Fig. 6). The microprocessor system consists of the processor itself, programme memory, data memory, the non-volatile parameter memory, and electronics that connects the analyzer to the keyboard. The control unit consists of a six-digit display and keyboard. The information messages and results are printed out by a thermal 20-character built-in printer. The results can also be transmitted to a computer via a serial interface. The measurement electronics and the wavelength selection are controlled by the microprocessor system.

The analogue system described in Figure 6 was also developed with simple electronics and at low cost for accurately measuring low intensities from several channels. Regardless of the intensity of the light passing through the liquid in the cuvette, which depends on the source of the light, the optical system and the quality of the cuvette as well as the wave-

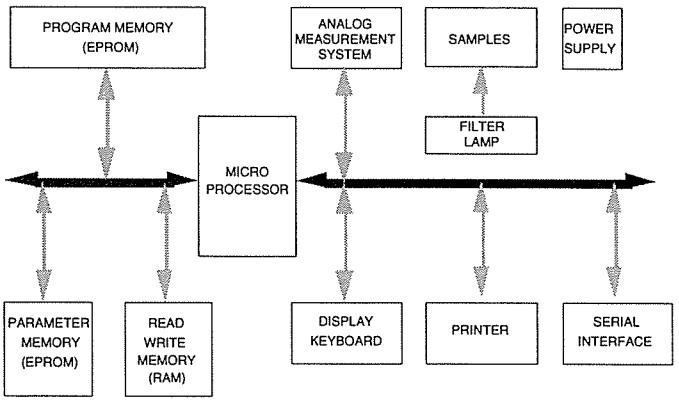

Figure 6. Functional diagram of the electronics of the FP-901 Analyzer.

length, the analogue system makes it possible to measure the same sample with high accuracy and with high or low light intensities. This feature is especially important when measuring samples at 340 nm with low light intensity

Fig. 7 describes the main mechanical parts of the analyzer. The front of the analyzer is equipped with a track along which a cuvette block tray slides. The measuring head is above the track. The cuvette block tray slides under the measuring head and allows the vertical measurement to be carried out. On the right is the main control panel with push-buttons for operation, the digital display, and the printer. On the rear panel the analyzer has connectors for auxiliary equipment and the serial interface.

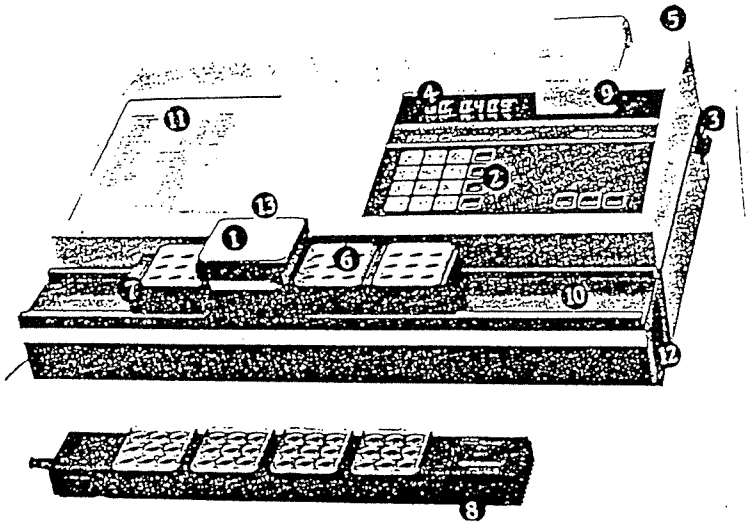

Figure 7. Front view of the FP-901 Analyzer.

1. Measuring head
2. Controls:
   2a. keyboard
   2b. power switch
3. Digital display
4. Rear panel
5. Cuvette block
6. Cuvette block tray
7. Cuvette block holder
8. Printer
9. Feeding track
10. Place for instruction sheet
11. Air filter
12. Cover locking screw

A4. TEST RESULTS AND APPLICATIONS

In this experimental section the theory of vertical photometry will be tested in practise, using various closely interrelated photometers (FP-9, FP-901, Multiskan). Comparisons are made between conventional horizontal and vertical photometry, with analysis of the advantages and disadvantages of both.

A4.1. *Sources of errors in photometry*

The most common errors that affect measured absorbance values stem from variations in the pathlength of the light in a sample, temperature variations, molar absorptivity and sample and solvent volume. Often related to parameters that markedly affect absorbance measurements in terms of their accuracy, precision and linearity, are parameters, such as stray light, bandwidth, wavelength accuracy and precision, and noise and drift in the measuring instrument. In addition, there may be variations in sample and cuvette handling, improper mixing, poor pipetting, infrequent calibrations of both optical and temperature control parameters. The lack of proper standard reagents and recording units also cause errors in absorbance measurements (Lewis, 1978).

A4.2. *Evaporation and temperature*

When small volumes are used, evaporation of the reaction mixture in a cuvette will contribute to a significant analytical error. Only a few authors have concerned themselves with this problem (Czarnetsy et al., 1970; Ferguson, 1966; Burtis et al., 1975; Burtis, 1976).

Table I shows that as the temperature of the liquid in the cuvette rises from 23.8 – 37.0°C, a three-fold increase in evaporation occurs. When the cuvette contained 750 µl of liquid, the liquid surface being 12 mm below the top of the cuvette, the extent of evaporation was significantly greater than from a cuvette with 375 µl of liquid and with a surface 17 mm below the top. In addition, condensation of the evaporating liquid is proportional to the distance of the liquid surface from the

Table I. Evaporation of water and serum mixture has been measured at two different volumes and temperatures in an FP-9 cuvette block made of nine cylindrical wells. The bottom area of each cylindrical well is 0.760 cm^2 and the depth is 22 mm. Each well contains 750 µl of liquid, which forms a column of about 10 mm. The amount of liquid in each well was weighed on a Mettler H10 W balance.

	Evaporation mg/30min			% / 30 min	
	Volume	Temperature, °C			
		23.8	37.0	23.8	37.0
		Mean ± SD	Mean ± SD		
1. Serum (90 µl) + H_2O (660 µl)	750	3.96 ± 0.26 (4)	11.25 ± 0.15 (4)	0.53	1.50
2. H_2O	750	3.96 ± 0.44 (4)	10.72 ± 0.42 (4)	0.53	1.43
3. Serum (45 µl) +H_2O (330 µl)	375	2.70 ± 0.16 (4)	8.02 ± 0.43 (4)	0.72	2.14
4. H_2O	375	2.66 ± 0.16 (4)	8.05 ± 0.25 (4)	0.71	2.15

( ) number of measurements.

top of the cuvette; the longer the distance thegreater the condensation. Further, the larger the free liquid surface area is the greater the evaporation. Because of these factors, evaporation must be considered when designing analytical devices for measuring analytes in liquid samples. A great deal of consideration must be given to the precise thermal equilibrium of any analytical system.

Other properties of analytical samples that also affect evaporation include: temperature (Table I), surface tension, diffusion coefficient, and concentration. Environmental factors include room temperature and relative humidity.

The rates of enzyme reactions strongly depend on temperature, which therefore must be maintained constant during the test procedure (Bergmeyer, 1973). That temperature must be controlled throughout the entire test period was previously demonstrated (Suovaniemi, U.K. Patent 1,499,414). The lack of an international consensus on temperature has created problems for both manufacturers and laboratories. In the opinion of the author 37°C should be adopted as a standard in determinations of enzyme activity in clinical chemistry. However, considerable differences of opinion exist as to the optimum temperature at which enzyme reactions should be measured (The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, 1974; Duggan, 1979).

It is also important to note that little attention has been paid to the problems of evaporation and leakage from specimen and reagent containers (Burtis et al.,1975; Burtis, 1976; Lewis and Wardle, 1978). In the FP-Analyzer systems the design is such that nine tubes are contained within one block with a cap plate, which prevents evaporation and leakage (Suovaniemi, U.K. Patent 1,392, 792).

A4.3. *Inaccurate pipetting of solute*

Various malfunctions and misuse of pipetting devices are some of the major sources of pipetting errors (Machin et al., 1973; Tiffany et al., 1976).

Table II shows that when the same amount of $KMnO_4$ is measured photometrically, dispensed into different volumes from 100 to 800 µl, i.e. at widely different concentrations, there is no significant difference in the measured absorbances. The small decrease in absorbance values seen with the FP-9 and FP-901 photometers, as the volume increases, is due to the added fact that the cuvette wells taper 10 arcminutes from top to bottom. This small deviation from an ideal cylinder is necessary for molding reasons in the production of the FP-cuvette blocks. This tapering does not occur in the microtitration plate wells used with the Multiskan instrument. In addition, on the inner walls of an FP-cuvette block, there are three vertical fins to guarantee proper mixing. When measuring the absorbance of a 200-µl solution, the optical effect of these fins is seen as a slightly higher absorbance, as compared with the result at higher volumes. The slight difference between the FP-9 and FP-901 photometers is due to the fact that different interference filters were used.

Based on these results, it is concluded, first, that the equation derived for the vertical measurement principle does (equation 13) indeed hold quite accurately, the absorbance being independent of the concentration of the measured substance to an accuracy of 1-2 parts in a thousand. This also means that, in practise, evaporation of the solvent from the cuvette, or inaccurate pipetting of the non-absorbing solvent, does not introduce analytical errors when the vertical light path measurement is used.

Table II. Absorbance (A) of potassium permanganate ($KMnO_4$) solutions at 540 nm measured with the Multiskan MC, FP-9 and FP-901 photometers, where the molar amount of potassium permanganate is constant, but its concentration is changed by adding various volumes of water. $A_{mean} = A_{KMnO4} - A_{H2O}$.

Volume µl	Multiskan MC	FP-9	FP-901
	A_{mean}[a] ± SD	A_{mean}[b] ± SD	A_{mean}[b] ± SD
100	0.187 ± 0.001		
150	0.189 ± 0.002		
200	0.191 ± 0.002	0.194 ± 0.001	0.197 ± 0.001
250	0.189 ± 0.001		
300	0.191 ± 0.002	0.191 ± 0.001	0.195 ± 0.001
400		0.189 ± 0.002	0.194 ± 0.001
500		0.189 ± 0.002	0.193 ± 0.001
600		0.188 ± 0.002	0.192 ± 0.001
700		0.188 ± 0.002	0.192 ± 0.002
800		0.187 ± 0.002	0.192 ± 0.001

a) means of 48 measurements
b) means of 18 measurements

A4.4. *Layering*

The effect of layering was studied by monitoring bacterial growth with a vertical beam photometer (Mutascreen, Suovaniemi et al., 1984) and with a conventional horizontal beam photometer (Shimadzu). In all experiments the *Escherichia coli* WP2 uvrA strain was used. The Vogel-Bonner culture medium was supplemented either with 1.5 µM or with 15 µM tryptophan. In the former case the bacteria grew to a final density of only $4x10^7$ cells/ml, but in the latter, the final density was $5x10^8$ cells/ml. In the Mutascreen instrument a wide band filter (400-700 nm) was used. In the horizontally measuring photometer the samples were scanned in the 400-700 nm wavelength range.

Fig. 8 demonstrates that cell growth is accurately measured with the vertical photometer (traces A and B). With the horizontal measurement, however, the absorbance increase because of the increase in cell number is counteracted by the layering of the cells towards the bottom of the cuvette (trace D). This phenomenon, entirely due to layering, as the bacteria are no longer multiplying, is evident from curve E. This difference between the vertical and horizontal measurements of the same samples is expected from the previous theoretical analysis. Thus, as the derivation of eqn. 13 from eqn. 12 showed (section A2.3.), since the total mass of absorbing substance is always contained within the column of liquid, the measured absorbance is insensitive to inhomogeneities in the solution due to layering, for example.

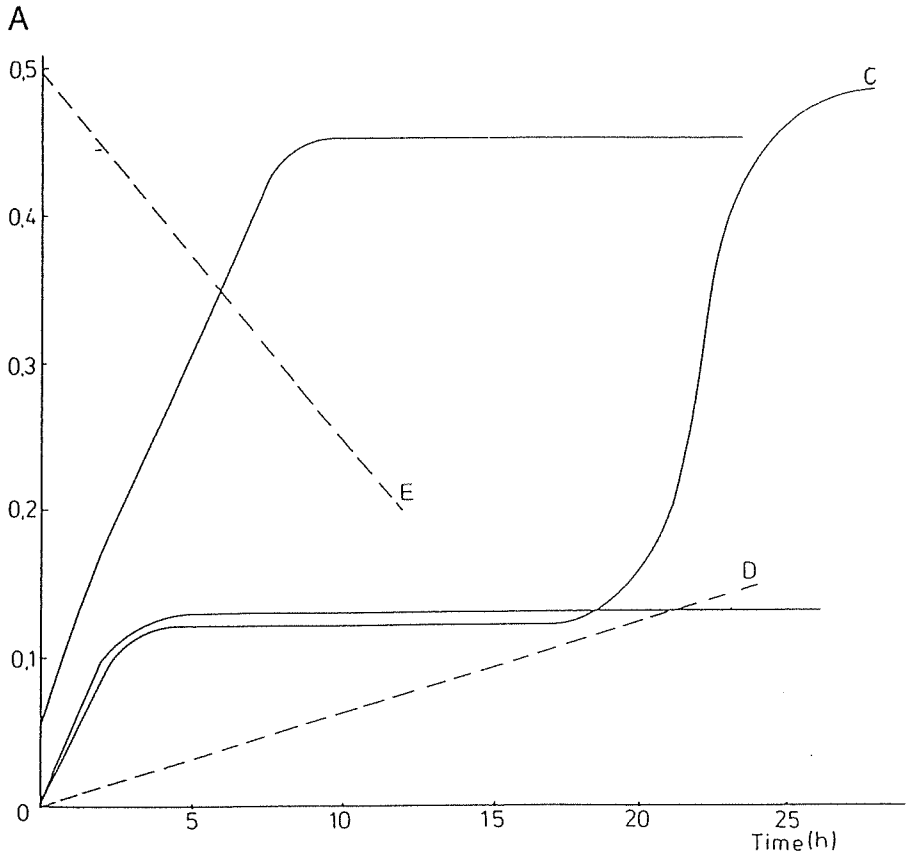

Figure 8. Schematic representation of growth curves of E. coli as measured with Labsystems' Mutascreen (A-C) and with Shimadzu (D-E) photometers.

Curves A-C show the change of turbidity as seen with Mutascreen and curves D-E show the change of turbidity as seen with the horizontally measuring photometer. Curves A and D show the growth of bacteria in the medium with 15 μM tryptohan, the final density of the cells in both cases is $5x10^8$ cells/ml. Curve B shows bacterial growth in the medium containing 1.5 μM tryptophan, which limits the final bacterial population to $4x10^7$ cells/ml. Curve C shows the growth of auxotrophic and prototrophic E. coli as seen in Labsystems' Mutascreen. Curve E shows the effect of sedimentation (layering) of bacteria as detected with a horizontally measuring photometer. The density of bacterial population is $5x10^8$ cells/ml.

A4.5. *Effect of the shape of the liquid surface in cuvettes made of polystyrene*

In polystyrene cuvettes, ordinary aqueous solutions produce a flat, even liquid surface. Under such circumstances the vertical light beam will leave the solution as if there were another optical window in the cuvette (Fig. 9a).

However, if the surface tension is lowered in the solution, a concave surface is produced (Fig. 9b). This is the case, for example, when protein or detergents are present. Figs. 10 and 11 show how this affects the absorbance in vertical photometry.

The pathlength for the central part of the light beam will, for the same volume of a liquid with concave surface, be somewhat shorter, and the measured absorbance smaller, as compared with a flat liquid surface (Figs. 10 and 11). The slope of the concentration–dependent curve will be lower (Figs. 11 and 12).

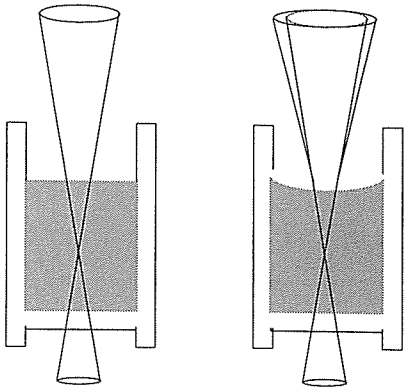

Figure 9.

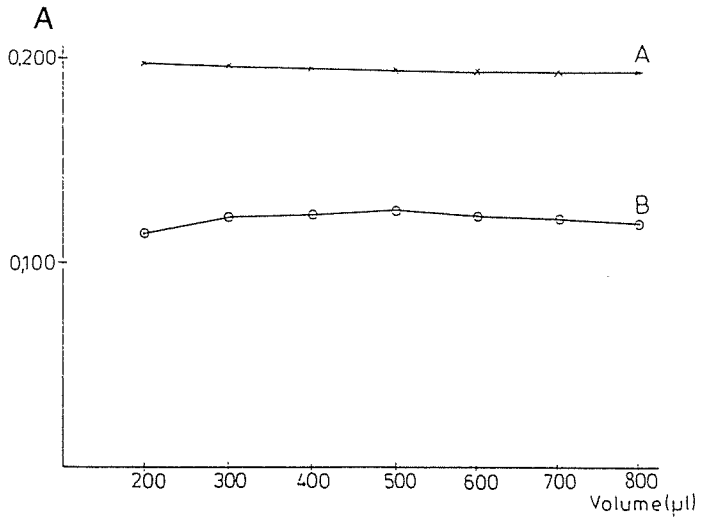

Figure 10. The absorbance of potassium permanganate ($KMnO_4$) measured with the FP-901 photometer at 540 nm. Into two cuvette blocks 200 µl of 0.3 mM $KMnO_4$ was pipetted, and water was then added in 100-µl aliquots. The instrument was blanked against a corresponding volume of diluent at each step. One cuvette block with 200 µl of 0.3 mM $KMnO_4$ served as a control

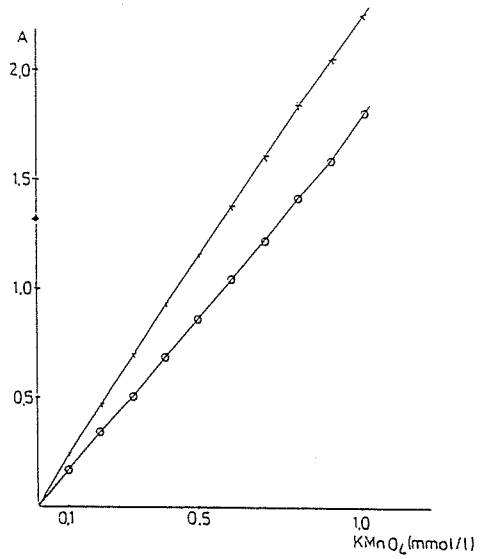

Figure 11. Absorbance of $KMnO_4$ measured with the FP-901 photometer at 540 nm, when the liquid surface is flat (water as solvent, x-x-x), and concave (0.1 % aqueous Tween 20 as solvent, o-o-o). Equal volumes of $KMnO_4$ at various concentrations were tested.

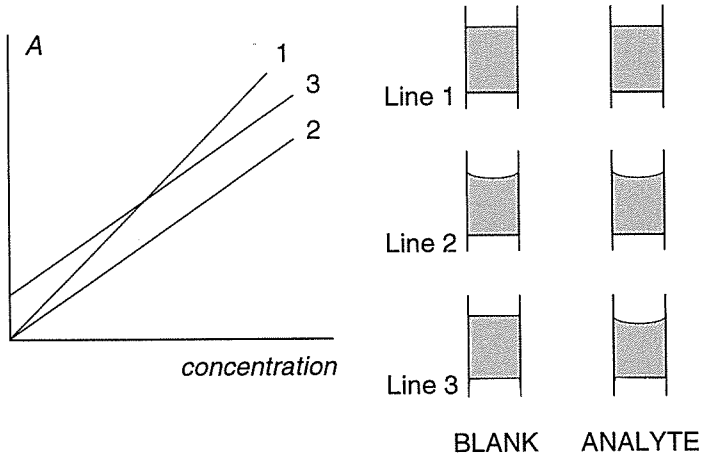

Figure 12. The effect of surface shape on absorbance vs. concentration curves.

Let us now consider light with an intensity I_o entering the optical window of the cuvette. The light passes through the liquid losing intensity because of absorbance. The intensity of light at the free surface is I, and the absorbance that depends on the concentration of the absorber in the liquid is

(14) $A = \log I_o/I$

With a concave liquid surface, some light reaching the periphery of the surface will be reflected away from the optical axis and will not reach the light detector (Figure 9b). The intensity of light measured by the detector, I_1, is thus less than that at the surface (I). The amount of light loss can thus be expressed as

(15) $A_1 = \log I/I_1$

The amount of this light loss occurring at the surface will, for all practical purposes, depend only on the shape of the surface, and not on the concentration or other properties of the solution. In the final absorbance measurement this loss will therefore appear as a constant additive term.

(16) $A_{tot} = A + A_1 = \log (I_o/I) + \log (I/I_1)$
$= \log (I_o/I_1)$

When performing actual absorbance measurements, the absorbance of a blank solution as well as that of the solution to be analyzed are measured, and the blank value subtracted. Thus,

(17) $A = A_{anal} - A_{blank}$

Both A_{anal} and A_{blank} consist of two parts, A and A_1 described above, and A_1 is the same in both. Therefore, A_1 will be eliminated in the blank subtraction procedure, provided, of course, that the surface properties of sample and blank are the same. Clearly, it is important that the shapes of sample and blank surfaces are kept similar in vertical absorbance measurements. If this is not otherwise achieved, then detergent may be added to create equally concave surfaces in blanks, standards and samples.

A4.6. *Performance of vertical light beam photometers*

Table III compares the accuracy of the FP-9 photometer and a Varian Cary 119 instrument.

Table III. A National Bureau of Standards (NBS) glass filter 3-269 (NBS Standards Reference Material 930a) was measured using the FP-9 and Cary spectrophotometers.

Wavelength nm)	A NBS	A CARY 118	A FP-9
404,7	-	0.534	0.528
440	0.525	0.527	-
465	0.482	0.484	-
488	-	0.507	0.501
514,5	-	0.517	0.517
546	-	0.502	0.497
577	-	0.524	0.525
590	0.547	0.542	-
600	-	0.548	0.543
632	-	0.543	0.539
635	0.546	0.545	

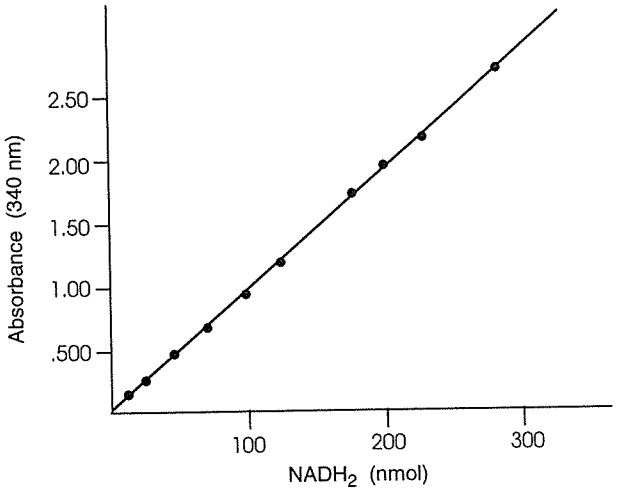

Figure 13. Linearity of the FP-9.

Each point is a mean of three measurements. $NADH_2$ was diluted to 750 ml of 0.1 mol/l Tris-HCl buffer, pH 8.0.

Fig. 13 reports the results from a test of the linearity of the FP-9 photometer at 340 nm. Each point shown is a mean of three measurements. Similar results were obtained at 546 nm (not shown).

Table IV demonstrates the inter- and intrachannel reproducibility of the FP-9 photometer. Note that this test, as the one in Fig. 13, was performed with a coloured solution, which was transferred to the cuvettes with a nine-channel pipette. Thus, the variability seen includes pipetting errors as well as errors arising from differences between wells in a cuvette block.

Table IV. A solution of hexamminecobalt(III)sulphate, $[Co(NH_3)_6]_2 (SO_4)_3 \cdot 5H_2O$, was measured at 500.6 nm several times (n=44) at the nine different channels on the FP-9 photometer. The mean absorbances and standard deviations are reported for each channel.

Mean ± SD		
1. 0.369 ± 0.002	4. 0.369 ± 0.002	7. 0.371 ± 0.002
2. 0.367 ± 0.002	5. 0.368 ± 0.003	8. 0.369 ± 0.003
3. 0.367 ± 0.002	6. 0.371 ± 0.002	9. 0.369 ± 0.002

The stability of the FP-9 photometer was tested using a glass filter (Corning no. 5330) and with a coloured solution. The hour-to-hour variation was found to be less than 0.001 absorbance units with the glass filter. With the coloured solution in place of the filter the variation was similar to that reported above (Table IV), i.e.less than 0.003 absorbance units.

As shown in Table V, the stability of the FP-9 photometer during the two-week performance period was better than 0.010 absorbance units.

Table V. The FP-9 photometer was tested by placing the NBS filters (SRM 930a) 1-269 and 3-269 in the light path and taking absorbance measurements at 406.6 nm, 13 times during a two-week period (9 x 13 values).

Filter	mean (n=9x13)	SD	CV%	lowest value	highest value
1-269	1.083	0.004	0.4	1.078	1.086
3-269	0.536	0.002	0.4	0.533	0.538

The performance of the FP-900 Analyzing system was evaluated in a multi-centre study according to the principles outlined in the 1st draft of the Standard for Instrument Testing, European Committee for Clinical Laboratory Standards (ECCLS). As examples of typical methodologies, cholesterol determination was selected as a typical end-point absorbance method, creatine kinase as a fixed-time (two-point) kinetic method, and aspartate aminotransferase as a multi-point, continuous method. The intra- and inter-laboratory imprecisions, the deviation from assigned values of commercial control sera, and carry-over were determined. The performance was compared with two other analytical systems, the Mark I Autoanalyzer and the Hitachi 705. The cost, speed and capacity were also estimated from the results of this study (Saris et al., 1983).

A5. CONCLUSIONS

The results from the studies reported above show that the characteristic performance of the vertical light beam photometers corresponds well to international standards set for photometers used in clinical and research chemistry. They also correspond well to what was expected from theory. The anticipated practical advantages of vertical photometry have also been concretised. Thus, the degree of automation in photometry has been vastly increased due to this development, and it is now possible to measure a large number of samples simultaneously, using relatively simple, low-cost equipment.

The principle of vertical photometry has also allowed completely new applications of the photometric technique that were not possible with a horizontal light beam. Some of these were indicated above, e.g. measuring the growth of micro-organisms that sediment in the reaction vessel. Such measurements may be extended to growth assays of bacteria (Suovaniemi et al., 1984), and various measurements of cell agglutination (Janatuinen et al., 1985; Swank et al., 1991).

B. LIQUID DISPENSION WITH ELECTRONIC PIPETTES

B1. FORERUNNER: THE MECHANICAL PIPETTE

In both research and routine laboratory work the accurate measurement and dispensing of reagents, i.e. pipetting, are essential for the success of quantitative analyses. Only a couple of decades ago pipetting was carried out almost exclusively by suction using glass pipettes. Single- and multichannel mechanical pipettes with adjustable volumes were developed by the end of the 1960s and first manufactured at the beginning of the 1970s by Finnpipette Co (Suovaniemi, Finnish Patents 44069 and 44070).Several other comparable products manufactured, e.g. by Gilson Co in France, Eppendorf Co, in Germany and Nichiryo Co in Japan, soon followed. In short, a fundamental change was about to occur in the way liquids were dispensed, especially in research and routine clinical laboratories. Now, since the beginning of the 1990s, new mechanical pipettes are purchased, or used ones replaced, at a rate of more than one million units per year. Yet, mouth suction glass and plastic pipettes are still used in many laboratories, although their accuracy and precision are usually inferior and, moreover, dependent on the skill and care of the user. In addition, their use is linked to considerable accident and health hazards.

In the 1970s the quality of both single- and multichannel pipettes, and the disposable pipette tips that they required, was quite variable. Wenk and Lustgarten (1974) noted, for example, that a pipette with a particular volume setting yielded different volumes during pipetting, depending on whether a used or unused pipette tip was employed. Zwart (1974) found that, depending on the manufacturer, most pipettes showed considerable calibration errors. Such initial difficulties were soon overcome, however, with further experience, research and development in this area, and improving the quality of both pipettes and pipette accessories. The best results were naturally achieved by those manufacturers, who in order to ensure complete compatibility between pipettes and pipette tips, developed them at the same time. These manufacturers were able to control the production quality of both products.

At this time, and after two decades of development, mechanical pipettes exhibit excellent accuracy and precision, far beyond that normally attained by classical mouth suction (section B4.). Yet, results with mechanical pipettes are still user-dependent, although not to the same degree as with the mouth suction pipettes. Experience and skill are still required by the user to reach high-quality results, and a significant number of laboratory personnel never acquire this, despite training.

Dispensing with the mechanical pipettes requires pressing a knob with the thumb, which initiates the outward movement of the plunger. Aspiration by mouth is replaced by releaving thumb pressure, which in turn mechanically causes inward movement of the plunger. In particular, the latter movement of aspiration requires both care and patience, because it must not occur too quickly. If it does, especially with volumes exceeding 0.1 ml, aspiration of air bubbles replacing some of the sample volume often results, yielding significant errors.

One might think that the present-day growth of automation and simplification, especially in clinical routine laboratories, would have made manual pipetting redundant. However, this is not the case. On the contrary, manual pipetting has, if anything, continued to increase, probably in part because mechanical pipettes are readily available. As described above, automation of light absorbance measurements, for example, has

been accompanied by the use of microtitration plates. Devices performing all the necessary pipetting automatically in such assays are relatively uncommon, expensive, and often limited to particular assays. Moreover, despite automatic dispensing of some reagents, they often still require additional manual dispensing (often the critical dispensing of the patient sample). In practise, this has meant that instead of pipetting into individual vials, multichannel mechanic pipettes are used, by which one may aspirate and dispense from or into as many as twelve vials (or reaction wells) simultaneously. This development has clearly increased the number of assays that may be performed daily in a single laboratory, and has simultaneously enhanced precision and accuracy. However, it has also brought with it some serious problems that may not have been anticipated during the relatively fast revolution of the working procedures in the laboratory.

Excessive use of multichannel mechanical pipettes today has simultaneously resulted in increased stress on laboratory personnel. Multichannel pipettes, especially, require a strong force with the thumb to enforce pipette action. If this force is repeatedly exerted many times per working day, it may cause occupational disease. Burns and Johnson (1991) have recently reported that such repetitive performance may cause the so-called carpal tunnel syndrome, an inflammatory response to excessive physical stress on the hand and the fingers.

B2. DEVELOPMENT OF ELECTRONIC PIPETTES

From the above, it is clear that although mechanical pipettes revolutionized practical laboratory work, making it possible to handle larger assay volumes at higher precision and accuracy, some unforeseen drawbacks have accompanied their use. It became evident that the main required improvements centered on (i) making a device less dependent on human performance, and (ii) relieving the user from the physical stress that is associated with high volume mechanical pipetting.

One solution to this problem would be fully automated devices that would carry out all the dispensing and the measurements as well. As mentioned already, although such "robots" are available, they still have not made manual pipetting redundant. In fact, they are not expected to replace manual pipetting in the near future for several reasons. First and foremost, such automatic devices are very costly and can therefore not offer a world-wide solution for a long time. Second, due to the different requirements of individual assays in the laboratory, fully automated devices can, at best, handle only part of the work load. Finally, much laboratory work still requires constant visual human control. This is especially true for any assay of a more experimental or developmental nature.

Another solution would be to develop an electronically controlled pipette that would otherwise be analogous to the mechanical device in terms of size, weight, accuracy and precision, but in which the plunger would be activated electronically, following only a light touch. Such a pipette was described in 1975 (Appendix IV). However, the device described in this patent was never manufactured. Nine years later, in 1984, an American company, Rainin Instrument, Inc., revived this idea by applying for patents for an analogous device (Magnussen et al., U.S. Patents 4,671,123 and 4,905,526). Evidently, these later patents were granted because they were not in the same classification as U.S. Patent 4,058,370 (Appendix IV).

It is particularly interesting that Rainin Instrument Inc. has used a specific technical innovation, namely a so-called "floating plunger connector, located periferally with

respect to the plunger long axis (Appendix IV) in manufacturing their mechanical and electronic multichannel pipettes. Since the plungers can move to the side, the pipettes function with low friction and are easy to use.U.S. Patents 3,855,868; 4,058,370 and 4,215,092 (Suovaniemi) describe this innovation.

The development of electronic pipettes, and the technical solutions required for this have thus taken a long time. This is so despite the well-known fact that scientists have desired to develop such devices for more than a decade at least. Rainin Instrument, Inc.'s single and multichannel electronic pipettes possess good precision and accuracy (section B4) and function without having to exert excessive force. However, compared with the mechanical forerunners, they are much larger and heavier. Furthermore, a new unforeseen problem has arisen with these pipettes: the operator feels a lack of control. Apparently, it is essential for the user to "feel" he is controlling the dispensing procedure, as is clear when using a mechanical pipette. The Rainin electronic pipettes are simply too slow-reacting to meet this requirement, i.e. the response time between pushing the activator and the plunger movement is too long, at least for many experienced laboratory workers (section B4).

Another product of this kind was also initiated in the 1980s, dating from 1985 (Tervamäki, Finnish Patent application 852704).This pipette works with an electric motor, with the volume adjusted manually by means of a screw. The product is at present manufactured by Labsystems Oy in Finland. However, it has a major drawback; it lacks a secondary plunger movement during dispensation, which is a prerequisite for achieving acceptable precision and accuracy in pipetting, as described by the author two decades ago (Suovaniemi, Finnish Patents 44069 and 44070). Performance data on this pipette are reported below (section B4).

Based on the above, it is concluded that up to the 1990s the developed electronic pipettes have either not met the standards set originally, or have added new problems that were not anticipated during development. Apparently, and perhaps surprisingly, paramount difficulties have been encountered in developing satisfactory electronic pipettes. The fact that at present the leading manufacturers of mechanical pipettes (Gilson in France and Eppendorf in Germany) have yet to produce their own portable electronic pipettes attests to these difficulties.

B3. THE BIOHIT PROLINE ELECTRONIC PIPETTE

Table VI gives a comparison of the size and weight of the Biohit Proline Electronic (BPE) series pipettes, microprocessor-controlled electronic devices, and the Rainin electronic pipette.

Table VI.

	Weight (g)	Length (cm)
BPE	144	24
Rainin	244	29

It is noteworthy that the BPE devices and their manual counterparts are of comparable weight and size, whereas the Rainin pipettes are considerably heavier and bulkier.

Compared with the manual pipettes, the BPE pipette, as defined by the function of the microprocessor, clearly possesses more advantageous properties particularly with respect to long-term precision (section B4). and ease of use. These properties become accentuated in a long pipetting series, or in dispensing programmes. Even a complicated, well-defined, dispensing series can be carried out by adjusting the software that drives the microprocessor. This makes the pipette suit-

able for use with specialized diagnostic kits, which often require specific dispensing programmes.

The main elements distinguishing the BPE pipettes from their manual forerunners may be described as follows: A battery makes cableless functioning possible; a motor performs the actual pipetting, i.e. moves the plunger; the control unit, consisting of six control push-buttons and a display, is used to choose different functions and parameters. The microprocessor steers the control unit and the motor. The pipette's separate charging device, securing long-term functioning, serves as a pipette stand so the batteries are automatically recharged when the pipette is not in use (Fig. 14).

In choosing the battery, size, charging and discharging properties, connector voltage and lifetime were emphasized. A cordless charging system was designed on this basis (Suovaniemi and Ekholm, European Patent application 91304061.4).

A DC motor best satisfied the most important criteria of the motor: small size, low price, low-power consumption, high acceleration, and rotation velocity. A small stepping motor could have been an alternative, but it is more expensive than the DC motor, which is also more favourable with respect to power usage, maximal speed of rotation, torque, and availability.

The DC motor is limited in its inability to be controlled precisely, especially when the controlling electronics require small current usage and very small physical size simultaneously. Thus, it was not possible to implement a conventional and complicated steering electronics because of the limitations conferred by physical size, current consumption, the need for a powerful microprocessor and pricing. For these reasons the control of the DC-motor is based on the use of a mechanical element (Suovaniemi and Ekholm, European patent application, PCT/FI 91/ 00133). With this patented method, a small, 4-bit microprocessor (made by NEC Electronics, Japan) can be used to further reduce the cost. A pinion has been linked to the shaft of a plunger-associated screw, so that the pinion and shaft can rotate. Two optical readers control the rotation of the pinion and at a point corresponding to the decided length of

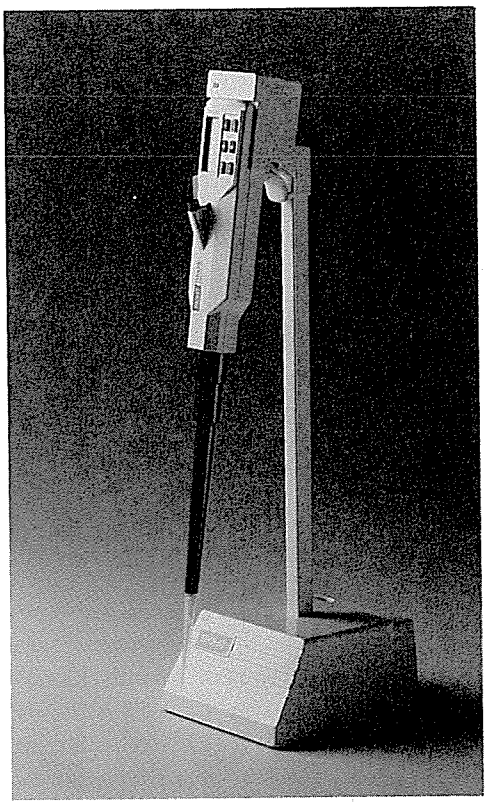

Figure 14

The basic Biohit Proline Electronic (BPE) pipette. The battery and the DC motor are in the upper and lower ends of the handle. The control unit is composed of control push-buttons and a display. The charging device also functions as a pipette stand.

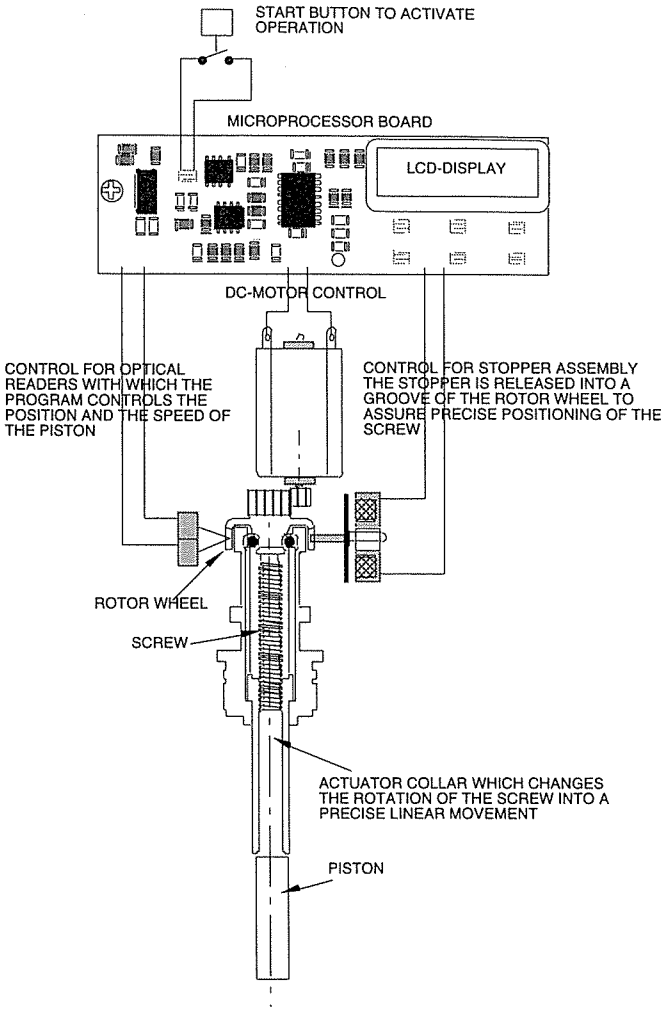

Figure 15. Operational principle of the electro-mechanics of the BPE pipette.

ment, the pinion is inserted into it. The length of movement, at certain stepping increments, is therefore highly accurate. The deceleration of the movement is very fast, an added feature of this new method. In the dispensing mode this means a sharper cut of the liquid stream at the end of the tip resulting in higher precision and ease of use.

The steering of the motor is based on optical feed-back from a disk with five equally-spaced peripheral cogs and a coplanar mirror. The microprocessor controls the number of steps, which correspond to a certain length of plunger action. The speed of the plunger movement can be regulated by adjusting the current of the DC motor. When the velocity, or rather the velocity profile, chosen by the user, has been achieved, it is implemented by the microprocessor-steered optical feed-back (Appendix V).

Since the pipette controls the movement of the piston, the laboratory's most frequently used pipetting operations, i.e. pipetting, multiple dispensing and dilution, can be

carried out with it.

Figure 16a illustrates the various phases of the basic pipetting cycle. The aspiration of the liquid starts from the home position (H) and for a velocity profile the piston is linearly accelerated in order to avoid foam and bubble formation in the liquid. When the desired amount of liquid is aspirated, the stopper is activated to ensure the correct linear movement of the piston (black dot in the drawing).

As the liquid is dispensed, the piston is driven back to the home position, and the movement is continued through the blow-out area to the zero level (0). This additional piston movement ensures that no liquid remains in the tip. After a short pause, the piston is automatically driven back to the home position and is ready for the next operation.

The multiple dispensing (Fig. 16b) has the same basic functions, but the dispensing cycle is divided into preprogrammed dispensings of the aliquots. It is to be noticed that the volume aspirated is greater than the volume needed for the aliquots. Before the first aliquot is taken, a reset dispensing is performed as some liquid is left in the tip from the dispensing of the last aliquot. This ensures that each aliquot dispensing has similar conditions, which leads to greater accuracy and precision.

Because of the dilution mode (Fig. 16c), two different volumes of liquid can be aspirated into the tip and then simultaneously

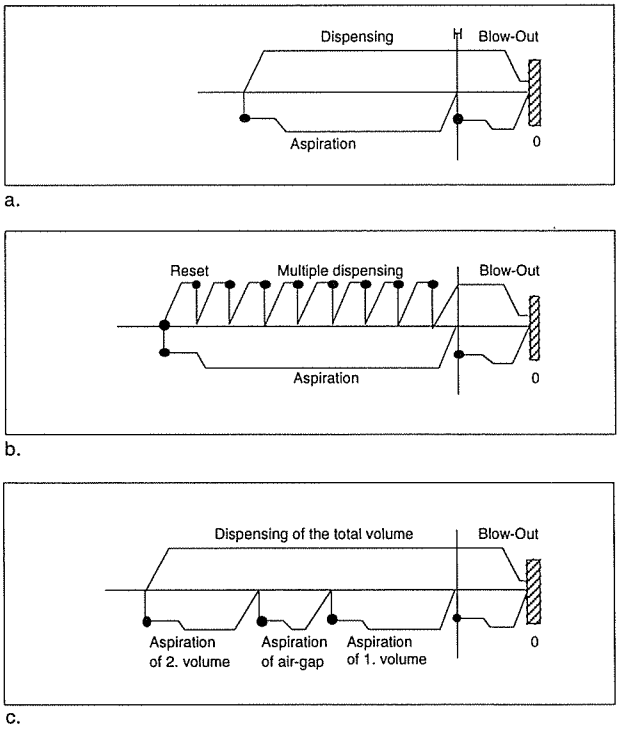

Figure 16. Procedure for filling and emptying the BPE pipette (for details, see text and Appendix V).

dispensed. An air gap between two liquids is automatically added in order to avoid contamination when aspirating the second liquid.

The speed of the piston movement can be individually programmed for each operation mode.

Velocity profiles make the functioning both smooth and pleasant and favour optimization of the liquid laminar flow when using pipette tips of various shapes and liquids of different viscosities. The secondary movement through the blow-out area, which always ends the dispensing phase, is essential for complete emptying of the pipette tip (see section B4).

The control unit is composed of 6 control push-buttons and a display. For reasons of economy, a microprocessor that already contained an integrated, liquid crystal display (LCD) driver was chosen. This made it possible to use a low-current LCD display. Both the six-button control unit and the starting button, which initiates motor performance, are based on an electronic solution that minimizes current usage. The separate starting button is positioned for optimal ergonomy; it is activated by a light push with the thumb. The microprocessor registers the change in the circuitry caused by activating a push-button, using the conventional technique of short-circuiting specific parts of the electronics.

The entire electronics have been designed by implementing the surface mount technique, which has made it possible to achieve a resting current of only about 1 mA. This prolongs the lifetime of the charging system. The work capacity, without recharging, depends on the volumes, plungers, and number of channels used. Several hundred pipettings can be carried out with the large multichannel pipettes, but with the one-channel versions, this number may reach 5000.

For physical reasons, the aspirated and dispensed volumes are not linear functions of the mechanical movement of the plunger over a large range of volumes. The corrections for this have been done either by adjusting the microprocessor step number, or by non-linearizing the screw. The non-linearity, especially in the small-volume range, can be easily corrected by modifying the screw threading (Appendix VI). However, when the volume range is large (e.g. 0.5 – 5.0 ml) compared to the resolution of the mechanical movement, corresponding to e.g. 10 µl of liquid, correction can be done under microprocessor control.

Plunger sealings are of special importance in a product of this kind. Tightness, long life-time, endurance towards torque and vibrations, and stability of quality are special requirements. These must, moreover, be combined with especially low frictional properties. A recent patent application (Suovaniemi, European patent application, PCT/ FI 92/00075) presents some possible solutions for the above problems.

B4. TEST RESULTS

Pipette performance was tested by pipetting water samples to a vial or a sponge on a sensitive balance (Ohaus 6A 200 D, Ohaus Corporation, U.S.A.). The balance was connected to a computer; weight readouts and statistics were obtained using BIOHIT/ Balance Reader software.

Certain precautions had to be made in pipetting volumes below 25 µl. Dispensation with both the Rainin and Labsystems pipettes was often found to be incomplete, as a small volume remained in the tips. This was especially common when using slow pipetting speeds. This phenomenon was not observed with the BPE pipettes, presumably due to their effective secondary plunger movement. For this reason, all pipettings below 25 µl were performed into a sponge by lightly

touching the sponge with the pipette tip. Hence, different pipettes gave comparable results. Larger volumes were pipetted into a vial, without touching it with the pipette tip.

Pipetting speed was tested separately for each device, using both fast and slow speed settings on the pipette.

The following pipettes were tested: Multichannel BPE pipettes (12-, 8-, and 4-channels; both the 100-µl- and 250-µl versions were tested for each), single channel BPE pipettes (10-, 100-, 250-, 500-, 1000- and 5000-µl versions), and, for comparison, Biohit manual pipettes (10-, 50-, 200-, 1000-, and 5000-µl versions).

Analogous tests were also performed using the Rainin single channel pipettes (10-, 25-, 100-, 250- and 1000-µl versions), the Rainin 8-channel, and the Labsystems FP-Electronic pipette.

Each pipetting series comprised ten consecutive pipettings at a single volume setting with each pipette (or pipette channel). Three volumes were tested for each pipette, at the 100, 50 and 10% levels (or at the low-volume limit) of the pipetting capacity. For multichannel pipettes each channel was first tested, after which channel 1 was further tested by four series of pipettings (each with n=10).

B4.1. *Precision and accuracy of pipetting*

Tables VII - X summarize the data with the tested single-channel pipettes. It is interesting to note, first, that the performance of the BPE pipettes and their manual counterparts is comparable. However, the accuracy of the latter in the low-volume region is somewhat better, but this difference vanishes when volumes >50 µl are used. In this connection it must be emphasized that the reported results of manual pipetting are optimal, because the pipetting was performed by an experienced technician. Nevertheless, the electronic pipette showed, for example, a mean imprecision of 6.14% (range: 7.73 to 5.49) and a mean inaccuracy of -3.22% (range: -1.81 to -5.82) at the minimum volume of 1 µl. This must be considered acceptable at this very small volume. Both accuracy and precision improved considerably with increasing volume (Tables VII - X).

Comparison with the Rainin single channel electronic pipettes (Table VIII) and the Biohit Proline manual pipettes (Table IX) indicates that these have an accuracy and precision comparable to the BPE's. However, the Labsystems electronic pipette was clearly inferior, especially at volumes up to 100 µl (Table X).

Table VII. The Biohit Proline electronic pipette (single channel). Each value is a mean of 10 measurements of distilled water at +22°C.

	Volume (µl)					
	1	5	10	25	50	100
imprecision (%)	6.14[1)]	1.1[1)]	0.73[1)]	0.26[3)]	0.21[2)]	0.09[2)]
inaccuracy (%)	–3.22	–1.25	–0.49	–1.23	+0.24	–0.10
imprecision (%)			0.42[2)]		0.22[3)]	0.17[5)]
inaccuracy (%)			–1.21		-0.33	+0.21

	Volume (µl)					
	125	250	500	1000	2500	5000
imprecision (%)	0.11[3)]	0.06[3)]	0.06[4)]	0.05[5)]	0.07[6)]	0.12[6)]
inaccuracy (%)	–0.43	–0.37	–0.49	+0.29	+0.09	–0.02
imprecision (%)		0.06[4)]	0.07[5)]			
inaccuracy (%)		–0.29	+0.22			
imprecision (%)			0.13[6)]			
inaccuracy (%)			+1.21			

Different models of the Biohit Proline electronic pipettes with different volume ranges have been used: [1)] 0.2-10 µl, [2)] 5-100 µl, [3)] 10-250 µl, [4)] 20-500 µl, [5)] 50-1000 µl, [6)] 500-5000 µl.

Table VIII. The Rainin EDP2 electronic pipette (single channel). Each value is a mean of 10 measurements of distilled water at +22°C.

	Volume (µl)					
	1	2	5	10	12	25
imprecision (%)	6.19[1]		1.43[1]	0.74[1]		
inaccuracy (%)	−3.02		+0.51	+0.65		
imprecision (%)		3.08[2]			1.36[2]	0.91[2]
inaccuracy (%)		−4.82			−2.97	−2.36
imprecision (%)			1.03[3]			
inaccuracy (%)			−0.35			
imprecision (%)					0.36[4]	
inaccuracy (%)					−0.08	

	Volume (µl)					
	50	100	125	250	500	1000
imprecision (%)	2.89[33]	1.08[3]				
inaccuracy (%)	−0.19	+0.1				
imprecision (%)			0.14[4]	0.44[4]		
inaccuracy ((%)			−0.44	−0.50		
imprecision (%)		0.20[5]		0.05[5]	0.48[5]	
inaccuracy (%)		+0.40		+0.44	−4.25	

Different models of the Biohit Proline electronic pipettes with different volume ranges have been used: [1] 0.5-10 µl, [2] 2.5-25 µl, [3] 10-100 µl, [4] 25-250 µl, [5] 100-1000 µl.

Table IX. The Biohit Proline manual pipette (single channel). Each value is a mean of 10 measurements of distilled water at +22°C.

	Volume (µl)					
	1	5	10	25	50	100
inaccuracy (%)	6.35[1)]	1.25[1)]	0.96[1)]			
imprecision (%)	+0.99	+0.07	−0.15			
inaccuracy (%)		1.86[2)]		0.36[2)]	0.85[2)]	0.35[3)]
imprecision (%)		−1.21		+0.56	+0.73	+0.01

	Volume (µl)				
	200	500	1000	2500	5000
inaccuracy (%)	0.42[3)]				
amprecision (%)	+0.03				
inaccuracy (%)	0.14[4)]	0.17[4)]	0.09[4)]		
imprecision (%)	+0.36	+0.36	+0.13		
inaccuracy (%)			0.25[5)]	0.11[5)]	0.09[5)]
imprecision (%)			+0.57	+0.10	+0.22

Different models of the Biohit Proline manual pipettes with different volume ranges have been used:[1)] 0.5-10 µl, [2)] 5-50 µl, [3)] 50-200 µl, [4)] 200-1000 µl, [5)] 1000-5000 µl.

Table X. The Labsystems electronic pipette (single channel). Each value is a mean of 10 measurements of distilled water at +22°C.

	Volume (µl)						
	10	25	50	100	250	500	1000
imprecision (%)	2.03	0.61	0.65	0.74	0.57	0.21	0.10
inaccuracy (%)	+6.09	−3.17	−1.02	−1.67	−0.93	−0.94	−0.76

B4.2. *Multichannel pipetting*

It is important in multichannel pipetting that the variation in the performance of the individual channels be minimal. Fig. 17 shows results using the Proline 12-channel 100-µl pipette, at 25- and 50-µl pipetting volumes, respectively. It was found that in no channel did imprecision and inaccuracy exceed ±1.8% at 25 µl, and ±0.6% at 50 µl.

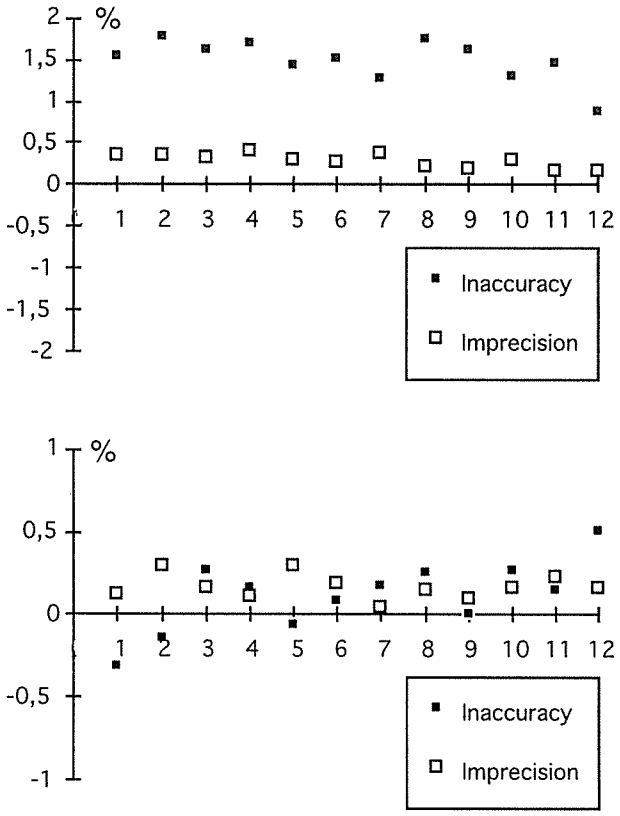

Figure 17. Biohit Proline electronic pipette (12-channel, volume from 5 to 100 µl). Tested pipetting volumes 25 and 50 µl, respectively. Pipetting was performed with distilled water at +22°C.

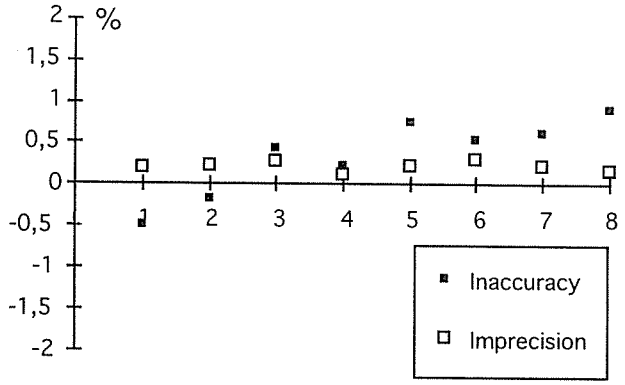

Figure 18. The Biohit Proline electronic (8-channel, volume from 25 to 250 µl) Tested pipetting volume is 25 µl. Pipetting was performed with distilled water at +22°C.

Fig. 18 shows the performance of the Proline 8-channel 250-µl pipette at 25-µl pipetting volume. Here the imprecision was +-1.5 % and the inaccuracy +-0.5%, when individual channels were compared. The 8-channel 250-µl Rainin pipette performed similarly (Fig. 19). As expected, the precision and accuracy consistently improved with increased volumes, also in the multichannel versions.

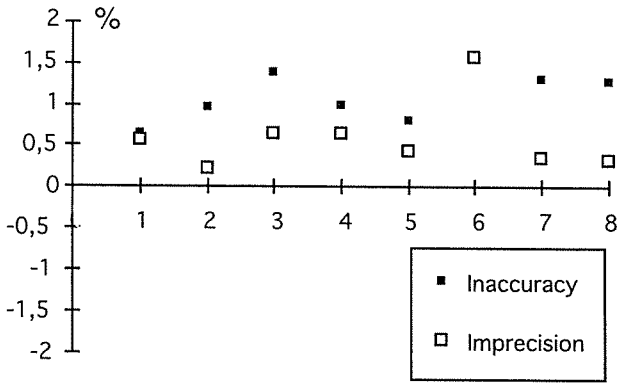

Figure 19. The Rainin electronic pipette (8-channel, volume from 25 to 250 µl). Tested pipetting volume is 25 µl. Pipetting was performed with distilled water at +22°C.

B4.3. *Pipetting speed*

The speed of pipetting was measured with different pipettes, at maximum volume of each. The total time taken to perform ten pipettings was measured. The BPE's are roughly three times faster than the Rainin counterparts, and the Labsystems' Electronic is of comparable speed or faster than the BPE's. A significant reason for the latter difference is the fact that the Labsystems pipette lacks the secondary plunger motion, which is common to the Rainin and BPE pipettes. The poor accuracy and precision of the Labsystems pipette stem primarily from the lack of this movement seen from the comparisons in Tables VII-X.

B5. CONCLUSIONS

From the series of tests reported above, it can be concluded that the BPE pipettes have achieved the goals that were set for their development. Their pipetting accuracy and precision are comparable with that of the corresponding mechanical pipettes, without being heavier or significantly larger in size. This means that the pipetting performance has remained comparable, and errors introduced by the (inexperienced) user have been minimized. Furthermore, pipetting is no longer associated with tiring physical hand and finger movements, typical for mechanical pipettes, and especially their multichannel versions.

Finally, these newly–developed pipettes have an important property, related to their speed, but less easily described. This is the user's feeling of being in control of the pipetting process. When the speed of pipetting becomes too slow, then the technician easily experiences irritation as the device is not "following" the user's pace. Obviously, slowness of the device beyond the user's capabilities will slow down the laboratory routine as a whole, relative to the working potential of the technical staff. This may, of course, be an important factor, especially in clinical routine laboratories, where hundreds of tests are performed each day.

C. GENERAL DISCUSSION

The inventions of the multichannel pipette and the principle of handling several samples and reagents simultaneously in colorimetric assays originated with research work carried out in 1968 (Suovaniemi, 1976; Suovaniemi, U.S. Patents 3,855,868 and 4,144,030). This was the basis of batch analyses in clinical chemistry. The combination of the multichannel principle and that of vertical photometric measurement made it possible to develop and manufacture new analyzing systems. The first of these were the FP-9 and FP-901 photometers, soon to be followed by the Titertek Multiskan and Multiskan MC systems described above.

The systems described in this work represent modular analytical measuring instruments that handle several samples batchwise throughout the procedure. Their design was based on the multichannel principle for both sample handling and measurement, combined with the vertical optical path of the photometer. As shown here, the principle of vertical photometry allows conversion of the well-known Bouguer–Lambert–Beer equation (A=abc) into a new form.

$$A = (\varepsilon / S)m$$

From this, an entirely new aspect emerges, i.e., that the absorbance no longer depends on the concentration of the light-absorbing substance, but on its molar amount. The experiments described above (section A.4) confirmed that this is indeed the case, also in practise.

This unique property of the vertical photometric measurement has proved useful in a number of ways. For example, inaccurate pipetting of non-absorbing solvents during an assay procedure, which obviously results in variations of the concentration of the absorbing substance, has no effect on the absorbance measured vertically. Evaporation of solvent (e.g. water) from the sample during the reaction measured, which also affects the concentration, will nevertheless not affect the vertically-measured absorbance. In addition, inhomogeneities in the solution, which may occur, for example, because of layering in turbidity measurements of bacterial growth, are also allowed.

On the other hand, the vertical measurement introduces a problem that is absent in horizontal measurements. Variations in the shape of the liquid surface in the cuvette will affect the measured absorbance (section A.4), and significant variations indeed occur in practise, due to changes in surface tension of solutions containing proteins or detergents. As shown above (section A.4), those potential errors can be easily overcome, however, by proper control measurements, and by making the surface tension in blanks, standards and samples the same.

It is essential that the bottom of the cuvette be optically homogeneous in order to get satisfying results with the vertical procedure. This problem is, of course, shared with the horizontal measurement, which requires optically homogeneous cuvette walls. Automation of the vertical technique has led to the development of so-called microtitration plates, and "strips", consisting of a number of polystyrene reaction wells. Developing these products to conform to the optical requirements of photometric measurements has been an important part of making general use of the vertical measurement principle.

The results of the present study showed that the characteristic performance of the developed photometer systems, with respect to accuracy, linearity, interchannel precision and stability, corresponds well with the standards set for such systems internationally (Saris et al., 1983). These developed instruments are suitable, for example, for clinical chemistry and enzyme immunoassays. These

instruments use either a block of cuvettes (FP cuvette block, Suovaniemi, U.K. Patent 1,486,210), microstrips or microtitration plates (Microstrip, Suovaniemi et al., U.S. Patent 4,319,841; see section A3 and Figure 4) for the measurements.

The development described here also included the first automatic photometric analyzer for conventional microtitration plates. Consequently, the use of the principle of a vertical light path has provided the basis for construction of small, flexible and cost-effective instruments of high sample capacity. As will be discussed below, such instruments have been developed for a large number of different purposes and applications.

The discovery of enzyme immunoassays, almost simultaneously by two research groups (Engvall and Perlman, 1971; Van Weemen and Schuurs, 1971), was foreshadowed by the coupling of enzymes to other proteins in histochemistry (Avrameas, 1969). Since then, the development of instrumentation described here, as well as of the proper solid phases in reaction wells for coupling with proteins (FP Cuvette block and Microstrip, manufactured by Biohit Oy), and the applications of enzyme immunoassays have continued to expand from research laboratories to every-day diagnostic purposes, especially in clinical microbiology.

From these studies development of sensitive fluorometers based on the principle of a vertical light path have come about. The theory, design, as well as immunological applications of such instruments, and related innovations, have been reported (Harjunmaa, U.S. Patent 4,758,523; Harjunmaa, 1986; Tiusanen, 1992). The vertical measurement was also the basis for the innovation of a new type of luminometer (Harjunmaa, U.S. Patent 4,678,326).

The FP-901 Analyzer was shown to be useful also for coagulation measurements (e.g. prothrombin time [PT], and activated partial thromboplastin time [APTT] with high throughput (100-200 samples per hour). It could further be adapted to chromogenic, clot and immunological EIA tests of the fibrinogen concentration in PT and APTT determinations (Janatuinen et al., 1985).

Another spin-off from the work described here was the Mutascreen instrument. The target was to develop an automated analyzer system for detecting mutagenicity and carcinogenicity, which overcame the drawbacks of the manual methods available (Suovaniemi et al., 1984; Falck, U.S. Patent 4,675,288). However, this attempt was not completely successful because the results of mutagenicity determinations in the liquid phase of bacterial growth did not in all cases correspond with the results of the manual Ames test, which is carried out in gel phase growth conditions (Ames et al., 1975). However, it is expected that further basic research in this area will eventually solve the problem of how to automate mutagenicity assays based on the vertical measurement principle (Suovaniemi et al., 1984; Gocke and Schübach, 1986).

The Mutascreen instrument was, in turn, further developed in two directions, the Bioscreen and the Auto-EIA Analyzer (both manufactured by Labsystems Oy). The Auto-EIA Analyzer is intended for automatic measurement of enzyme-immuno-assays (EIA). The applications of Bioscreen may be divided into two categories: automated studies with well-known test organisms, and studies on bacteria occurring in, or isolated from, different specimens. Bacterial growth is monitored by a vertical optical path turbidimetric system, in which the light beam passes the bacterial suspension through the bottom of the cuvette (see section A4.4). When the system monitors bacterial growth the turbidity readings are independent of the bacterial distribution in the cuvette, as well as of the volume of the solution (Suovaniemi et al., 1984). A further example of this measuring principle has been described by Bochner, who devised an automated technology for

identifying bacteria based on their metabolic capacity, using oxidation-reduction dyes (Bochner, 1989a, b).

Vertical light path photometers (e.g. Multiskan and Dynatech MR 580) can also reliably perform minimum inhibitory concentration (MIC) assays, which measure the lowest concentration of a drug that prevents visible growth of bacteria after incubation (Genta et al., 1982; Courcol et al., 1983; Turner et al., 1983). A diagnostic measuring system for bovine mastitis has also been developed (Sandholm, U.S. Patent 4,659,656; Mattila et al., 1986).

The unique properties of the vertical measurement principle might lead to new dynamic applications of photometry, in which the measured reaction does not lead to a homogeneous distribution of the measured absorbing substance in the measuring cuvette. Examples of such possibilities are the agglutination reactions typical in blood group determinations, other serological reactions, and determination of blood clotting (Suovaniemi, U.S. Patent 4,290,997 and 4,452,902).

Introduction of the multichannel pipettes and the Multiskan for microtiter plates and microstrips made it possible for scientists to accelerate the development of numerous enzyme immunoassay applications for research and clinical use. Microstrips as a solid phase in heterogenous enzyme immunoassays are used by major manufacturers of diagnostic test kits. For example, in practice, all laboratory diagnoses of HIV at the beginning of mass screening were made by instrument systems comprising vertical light path photometers, multichannel pipettes and microstrips.

D. FUTURE PROSPECTS

Today, the estimated sales volume of photometric measuring systems, multichannel pipettes, microstrips as well as the different auxiliary devices that have been developed on the basis of the vertical measurement principle, is one billion U.S. dollars per year. Although the basic discoveries on which this business is based, as well as the primary products, were made in Finland, only less than 10 per cent of these sales have remained in this country.

The Finnish companies, Labsystems and Eflab founded in 1971 and 1978, respectively, have sold several thousand FP-Analyzer systems and more than ten thousand Multiskans. The inventions of the multichannel pipettes (Suovaniemi, Finnish Patent no 44069), vertical photometry (Suovaniemi 1976 and 1984, Suovaniemi and Järnefelt 1982, Suovaniemi, French Patent 2,250,991, Appendices I-III) and microstrips (Suovaniemi et al., U.S. Patent no 4,319,841) were the bases for the very first microplate reader, the Multiskan (Suovaniemi, 1976 and 1984). From these inventions many other companies (e.g. Anthos, Beckman Instruments, Behringwerke, Bio-Rad System, Bio-Tek Instruments, Cetus, HP-Genenchem, Molecular Devices Corporation, Ortho Diagnostic System, Perkin-Elmer, SLT Labinstruments and Whittaker M. A. Bioproducts) have developed their own microplate readers as well as different auxiliary products.

The one- and multichannel electronic pipettes described in this work complement the earlier developments. In addition, further use of the technology of these devices can be made in the future in increasing the degree of automation of the measuring instruments. It appears likely that within the next five years, laboratories will be using electronic pipettes as commonly as they use mechanical pipettes now. It also seems probable that most of the present-day manufacturers of mechanical pipettes will develop electronic pipettes of their own.

Since the introduction of the single- and multichannel Finnpipettes, a number of companies (e.g. Costar, Gilson, Eppendorf, MLA, Nichiryo, Rainin and Socorex) have developed their own mechanical pipettes from these. Total annual sales of mechanical pipettes with disposable tips are 400 million U.S. dollars. It is probable, that within five years the electronic pipettes described here will encourage many companies to develop their own analogous to the worldwide markets, which may exceed one billion U.S. dollars by the year 2000. The innovations and development described here,as well as their applications, provide a starting point for creative research into further automation of laboratory analyses and development of new instrumentation.

E. ACKNOWLEDGEMENTS

It is great pleasure for me to express my sincere and respectful thanks to Professor Johan Järnefelt, M.D., former Head of the Department of Medical Chemistry, University of Helsinki, for supporting and encouraging my studies, innovations and product development.

I am also deeply grateful to Professor Nils-Erik Saris, Ph.D., Head of the Medical Chemistry Department, University of Helsinki, for his crucial advice and constant interest in my work.

Professor Mårten Wikström, M.D., Department of Medical Chemistry, University of Helsinki, has always readyly supported my work with invaluable guidance and helpful advice, which I greatly appreciate.

My warm thanks are due to Professor Herman Adlercreutz, M.D., Head of the Clinical Chemistry Department, to Associate Professor Matti Härkönen, M.D., Department of Clinical Chemistry, to Associate Professor Paavo Kinnunen, Ph.D., Department of Medical Chemistry, University of Helsinki, and to Mr. Kimmo Peitiläinen, M.Sc. (Theor. Phys), for their encouragement and inestimable help.

I wish to express my sincere and respectful thanks to Professor Ralph Gräsbeck, M.D., Head of the Medical Research Institute Minerva, Helsinki, and to Associate Professor Ilmo Hassinen, M.D., Department of Medical Biochemistry, University of Oulu, for their positive critique of the manuscript as well as for their inspiring ideas.

I am sincerely grateful to everyone who worked with me at the Finnish companies, Labsystems and Eflab, for their enthusiastic fidelity to my work.

I owe a great debt of gratitude to the personnel of the Finnish company, Biohit. Its creative atmosphere, like that at Labsystems and Eflab, where many of us have come from, continues to foster innovative ideas, which bear fruit in successful product development.

I am particularly grateful to my friend and President of Biohit, Mr. Paul Partanen, M.Sc., for his reliable and proficient work formerly at Labsystems and Eflab, and now at Biohit.

I am deeply grateful to Mr. Alpo Jokelainen (Tech., Phys.), Vice President of Biohit, Mr. Erkki Vesanen, M.Sc. (Electronics), Mr. Jukka Kilpiö, M.Sc., Raija Määränen, M.A., Ms. Sari Ylätupa, M.Sc., Ms. Raili Paasivuo, M.Sc., Mr. Kari Virtakare, M.Sc., Mr. Mikko Leskinen, Tech. and Mr. Tapani Miettinen, Tech. as well as Dr. Tapani Tiusanen, Ph.D., Dr. Hannu Harjunmaa, Ph.D., Mr. Kari Vauramo, Tech. and Mr. Pekka Peltonen, Tech., at Biohit, for their competent assistance.

My very special and warm thanks are due to my friend, Mr. Pertti Ekholm, Chief Product Designer at Biohit, with whom I have worked for more than two decades, first at Labsystems and Eflab and now at Biohit. He is a tireless innovator and exceptional product designer.

My gratitude is due to Sister Mary-Edel Lukko, o.c.d. who revised the English manuscript. I greatly appreciate all the assistance I received from Ms. Aila Koponen when preparing the manuscript for printing.

I owe much gratitude to Ms. Liisa Maria Kinnunen, M.Sc. who motivated and encouraged my exerting painstaking effort on the numerous manuscript drafts.

Words are insufficient in expressing my thanks to my parents, my wife Oili and my sons, Vesa, Joel and Ville, for their immeasurable patience and invaluable support.

This work was supported in part by grants from the Finnish development funds, SITRA, TEKES, KERA and Keksintösäätiö, which I greatly appreciate.

Helsinki, November 1993

Osmo Suovaniemi

F. REFERENCES

Al Moudallal, Z., Altschuh, D., Briand, J. P., and Van Regenmortel, M. H. V. (1984) J. Immunol. Meth., 68, 35-43.

Ames, B. N., McCann, J., and Yamasaki, E. (1975) Mut. Res., 31, 347-364.

Avrameas, S. (1969) Immunochemistry, 6, 43-52.

Bergmeyer, H. U. (1973) Z. Klin. Chem. Klin. Biochem., 11, 39-45.

Bochner, B. R (1989a) ASM News, 55, 536.

Bochner, B. R. (1989b) Nature, 339, 157-158.

Burns, K. P., and Johnson, R. R. (1991) Am. Biotech. Lab., 9, 20-21.

Burtis, C.A. (1976) Clin. Chem., 22, 1178.

Burtis, C.A., Begovich, and Watson, J. S. (1975) Clin. Chem., 21/23, 1907-1917.

Büttner, J. (1981) J. Clin. Chem. Clin. Biochem., 19, 529-538.

Cappel, R., de Cuyper, F., de Braekeleer, J. (1978) Arch. Virol., 58, 253-258.

Caraway, W. T. (1981) J. Clin. Chem. Clin. Biochem., 19, 491-496.

Cary, H. H. (1949) Rev. Sci. Instrum., 17, 558.

Chance, B. (1951) Rev. Sci. Instrum., 22, 619.

Commission of Enzymes of the International Union of Biochemistry Report (1961) London.

Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for determination of four enzymes in blood. (1974) Scand. J. Clin. Lab. Invest., 33, 287-306.

Courcol, R. J., Deleersnyder, H., Roussel-Delvallez, M., and Martin, G. R. (1983) J. Clin. Pathl., 36, 341-344.

Czarnetsy, E., Richeal, R., and O'Malley, J. (1970) Clin. Chem., 16, 521-522.

Dawes, E. A. (1972) Quantitative Problems in Biochemistry, 5th ed., Churchill Livingstone, Edingburg & London.

Deverill, I., and Reeves, W. G. (1980) J. Immunol. Meth., 38, 191-204.

Douillar, J. Y., Hoffman, T., and Herberman, R. B. (1981) J. Immunol. Meth. 39, 309-316.

Duggan, P. F. (1979) Clin. Chem., 25, 348-352.

Dwyer, D. S., Bradley, R. J., Urquhart, C. K., and Kearney, J. F. (1983) J. Immunol. Meth., 57, 111-119.

Eisenwiener, H.-G, and Keller, M. (1979) Clin. Chem., 25, 117-121.

Engvall, E., and Perlman, P. (1971) Immunochemistry, 8, 871-874.

Falck, K., U.S. Patent no 4,675,288.

Ferguson, G. A. (1966) Statistical Analysis in Psychology and Education, 2nd ed., McGraw-Hill, New York, 406.

Folin, O. (1904) Z. Physiol. Chem., 41, 223-242.

Freund, H. (1932) Colorimetry, Its Applications in Analytical and Clinical Practice, Scharfes Druchereien K.-G., Wetzlar.

Genta, V. M., McCarthy, L. R., Herrick, M., and Rose, F. (1982) Antim. Ag. Chemother., 22, 151-153.

Gocke, E., and Schüpbach, M. (1986) Mutation Res., 172, 1-9.

Granfors, K. (1979) J. Clin. Microbiol., 9, 336-341.

Granfors, K., Viljanen, M., Tiilikainen, A., Toivanen, A. (1980) J. Inf. Dis., 141, 424-429.

Gripenberg, M., Linder, E., Kurki, P., and Engvall, E. (1978) Scand. J. Immunol., 7, 151-157.

Gripenberg, M., Wafin, F., Isomäki, H., and Linder, E. (1979) J. Immunol. Meth., 31, 109-118.

Hardy, A. C. (1936) Handbook of Colorimetry, Technology Press, Cambridge, Mass..

Harjunmaa, H. (1986) Theory, Design and Immunological Applications of a Vertically Measuring Fluorometer, Academic dissertation, University of Helsinki.

Harjunmaa, H., U.S. Patents nos 4,678,326 and 4,758,523.

Hautanen, A., Koistinen, V., Penttinen, K., Wager, O. (1978) J. Immunol. Meth., 21, 335-340.

Iivanainen, M., Leinikki, P., Shekarchi, I., Taskinen, E., Holmes, K., Madden, D., and Sever, J. (1979) Cerebrospinal fluid antibody pattern in multiple sclerosis by ELISA., Transact. Neurol. Assoc., 104, 125-127.

Janatuinen, P., Tuominen, I., and Lalla, M. (1985) The report of the 3rd Asian-Pacific Congress of Clinical Biochemistry, 15-20 Sept., Bali - Indonesia.

Jemmerson, R., and Fishman, W. H. (1982) Anal. Biochem., 124, 286-292.

Keilin, D. (1925-26) Proc. Roy. Soc., s. B, xcviii, London, 312-339.

Keilin, D. (1966) The History of Cell Respiration and Cytochromes (prepared by J. Keilin), Cambridge Univ. Press, Cambridge.

Keller, H. (1978) J. Clin. Chem. Clin. Biochem., 16, 687-692.

Kendall, C., Ionescu-Matiu, I., and Dreesman, G. R. (1983) J. Immunol. Meth., 56, 329-339.

Kenner, C. T. (1973) In Instrumental and Separation Analysis, 6th ed., T. L. Brown, Ed. Charles E. Merrill Publishing Co., A. Bell & Howell Company, Columbus, Ohio.

Kobler, P. A. (1928) Photometric Chemical Analysis, 1, Colorimetry, John Wiley & Sons, New York.

Krebs, W. (1935) Klinische Kolorimetrie mit dem Pulfrich-Photometer, F. Volkmar, Leigzig.

Kurstak, E. (1986) Enzyme Immunodiagnosis, Academic Press, Inc., New York.

Köhler, G. and Milstein, C. (1975) Nature (Lond.), 256, 495-497.

Lansdorp, P. M., Oosterhof, F., Astaldi, G. C. B., and Zeulemaker, W. P. (1982) Tissue Antigens, 19, 11-19.

Lehtonen, O.-P. (1982) Characterization of the enzyme-linked immunosorbent assay (ELISA) with special refence to the avidity of measured antibodies. Academic dissertation, University of Turku.

Leinikki, P., and Pässilä, S. (1976) J. Clin. Path., 29, 1116-1120.

Leinikki, P., Shekarchi, I., Dorsett, P., Sever, J. (1978) J. Clin. Microbiol., 8, 419-423.

Lewis, S. A., and Wardle, J. M. (1978) J. Clin. Path., 31, 888-892.

Machin, E., Rohlfing, D., and Ansour, M. (1973) Clin. Chem., 19, 832-837.

MacMunn, C. A. (1885) Phil. Trans. Roy. Soc. of London, 177, 267.

Magnussen, H. T., Smith, C. L., Ruskewicz, S. J., Wingo, A. K., US Patents nos 4,671,123 and 4,905,526.

Manual of Optical Filters and Coatings (1983) Corion Corporation, 6-7.

Mattila, T., Syväjärvi, J. and Sandholm, M. (1986) Zent. Vet. [B], 33, 462-470.

McArthur, C. P., and Sengupta, S. (1981) J. Immunoassay, 2, 163-174.

Mellon, M. G. (1939) Present Status of Colorimetry, Industr. Engin. Chem., 11, 80-85.

Michaelis, L., and Menten, M. L. (1913) Biochem. Z., 49, 336-369.

Milatovic, D., and Braveny, I. (1980) J. Clin. Path., 33, 841-844.

Molnar, G. K., Viljanen, M. K., and Riekkinen, P. J. (1979) Acta Neurol. Scand., 60, 375-383.

Mondesire, R. R., Charlton, D. E., and Tizard, I. R. (1981) J. Immunoassay, 2, 45-57.

Myers, V. C. (1924) Practical Chemical Analysis of Blood, 2nd ed., Mosby Company, St. Louis, MO, USA,199-209.

Ruitenberg, E. J., Sekhuis, V. M., and Brosi, B. J. M. (1980) J. Clin. Microbiol. 11, 132-134.

Salonen, E.-M., and Vaheri, A. (1981) J. Immunol. Meth., 41, 95-103.

Sandholm, M., U.S. Patent No. 4,659,656.

Saris, N-E., Kansanen, M., Hekali, R., Huurrekorpi, L., Kivistö, H., Lehikoinen, T. (1983) J. Clin. Chem. Clin. Biochem., 21, 683-693.

Skeggs, L. T. (1957) Am. J. Clin. Path., 28, 311-322.

Suovaniemi, O., Canadian Patent no 1,031,183.

Suovaniemi, O., European Patent application 92/00075.

Suovaniemi, O., Finnish Patents nos 44069 and 44070.

Suovaniemi, O., French Patent no 2,250,991.

Suovaniemi, O., U.K. Patent nos 1,392,792; 1,486,210 and 1,499,414.

Suovaniemi, O., U.S. Patents nos 3,855,868; 4,058,370; 4,144,030; 4,215,092; 4,290,997; 4,439,039; 4,452,902.

Suovaniemi, O. (1976) Proceedings of the Second National Meeting of Biophysics and Biotechnology in Finland, A.L. Kairento, E. Riihimäki, and P. Tarkka, Eds., 183.

Suovaniemi, O., and Järnefelt, J. (1982) American Laboratory, June, 106-111.

Suovaniemi, O., Ekholm, P., and Partanen, P. (1982) U.S. Patent no 4,319,841.

Suovaniemi, O. (1984) Intl. Biotech. Lab., May / June, 43-45.

Suovaniemi, O., and Ekholm, P. (1991) European Patent applications, PCT/FI91/00133 and 91304061.4.

Suovaniemi, O., Ekholm, P., Falck, K., Kaukanen, E., Kinnunen, R., Partanen, P., Sorsa, M., and Vainio, H. (1984) Intl. Lab., 14, 52-61.

Suovaniemi, O., Ekholm, P., Järnefelt, J., Kaukanen, E., and Partanen, P., U.S. Patent no 4,452,902.

Suovaniemi, O., Kaukanen, E., and Ekholm, P., U.S. Patent no 4,431,924.

Swank, P. R., Lewis, M. L., and Damron, K. L. (1991) Thrombosis Res., 64 (2), 235-242.

Tervamäki, J., Finnish Patent application no 852704.

Tiffany, To. O., Thayer, P. C., Coelho, C. M., and Manning, G. B. (1976) Clin Chem., 22, 1438-1450.

Tiusanen, T. (1992) Inner-filter correction with a fluorometer-based multifunctional instrument. Academic dissertation, University of Helsinki.

Turner, A., Hawkey, P. M., and Pedler, S. J. (1983) J. Clin. Microbiol., 18, 996-998.

Ukkonen, P., Koistinen, V., Penttinen, K. (1977) J. Immunol. Meth., 15, 343-353.

Uotila, M., Ruoslahti, E., and Engvall, E. (1981) J. Immunol. Meth. 42, 11-15.

Vaheri, A., Vaananen, P., Salonen, E. M., and Suni, J. (1980) J. Clin. Path., 33, 845-847.

Van Weemen, B. K., and Schuurs, A. H. W. M. (1971) FEBS Lett. 15, 232-236.

Warburg, O., Christian, W., and Griese, A. (1935) Biochem. Z., 287, 157.

Wenk, R. E., and Lustgarten, J. A. (1974) Clin. Chem. 20, 320-327.

Zwart, H. (1974) Tijdschrift voor Medische Analisten, 29, 127-131.